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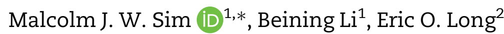
Review

Peptide-specific natural killer cell receptors



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Abstract

Class I and II human leukocyte antigens (HLA-I and HLA-II) present peptide antigens for immunosurveillance by T cells. HLA molecules also form ligands for a plethora of innate, germline-encoded receptors. Many of these receptors engage HLA molecules in a peptide sequence independent manner, with binding sites outside the peptide binding groove. However, some receptors, typically expressed on natural killer (NK) cells, engage the HLA presented peptide directly. Remarkably, some of these receptors display exquisite specificity for peptide sequences, with the capacity to detect sequences conserved in pathogens. Here, we review evidence for peptide-specific NK cell receptors (PSNKRs) and discuss their potential roles in immunity.

Keywords: NK cell; MHC-I; KIR; HLA-I; NKG2A/C

Introduction

The innate immune system uses germ line-encoded pattern recognition receptors (PRRs) to sense conserved molecular features associated with infections or cellular damage known as pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) [1]. Innate receptors such as NKG2D detect inducible ligands, expressed upon cellular stress caused by DNA damage, infection or transformation [2]. Natural killer (NK) cells are part of the innate immune system with important roles in immunity to cancer and viral infections [3]. Peripheral blood NK cells contribute to immune responses via cellular cytotoxicity and production of pro-inflammatory cytokines and chemokines [4]. A tissue resident NK cell subset, known as decidual or uterine NK cells (uNK) are also the dominant lymphocyte at the maternal-fetal interface and contribute to successful pregnancy [5, 6]. Activation of NK cells towards target cells is determined by integrating signals from numerous germline encoded activating and inhibitory receptors [4]. Like NKG2D, many NK cell activating receptors such as NKp46, NKp30 and DNAM-1 detect signs of cellular stress [7–10].

Another sign of cellular stress, infection or transformation, is loss of cell surface class I major histocompatibility complex molecules (MHC-I) [11, 12]. MHC-I molecules play an essential role in immune responses by presenting peptide antigens on the cell surface for immunosurveillance and detection by the T cell receptor (TCR) on T cells [13, 14]. NK cells also detect loss of cell surface MHC-I via MHC-I binding inhibitory receptors, summarized by the "missing-self" hypothesis [15]. However, many of these MHC-I binding inhibitory receptors exist as gene families that include multiple activating receptors such as the *Ly49*, *killer-cell immunoglobulin-like receptors (KIR)* and *NKG2x*. The function of MHC-I binding inhibitory receptors on NK cells is well-established, but the function of these activating receptors is much less clear, even counterintuitive. Some studies demonstrated these receptors exhibit weak or no binding to human MHC-I (known as HLA-I), perhaps indicative of a loss of function [16–19]. However, recent evidence suggests that some of these receptors are not weak but engage MHC-I molecules in a highly peptide-specific manner [20–24]. This new concept must be reconciled with the "missing-self" hypothesis as these receptors clearly require HLA-I expression for them to detect their ligands and stimulate NK cell responses. Further, the evolution of single germ-line encoded receptors that detect a limited repertoire of peptides in the context of a few HLA class I allotypes appears difficult to explain. The specificity and function of these receptors has been described on NK cells and we therefore term these receptors Peptide specific NK cell receptors (PSNKR). This property makes PSNKRs analogous to T cell receptors (TCR), however the role of PSNKRs in biology are largely unclear. Here we review evidence for PSNKRs and discuss their potential roles in immunity.

Peptide recognition discriminates MHC binding receptors

Cell surface expression of MHC-I depends on binding high affinity peptides [25] and in that regard, all MHC binding receptors are peptide dependent. However, MHC-I binding receptors can be further categorized by the direct contribution of bound peptide to the receptor-ligand interaction (Fig. 1). The TCR is the model peptide-dependent MHC-I binding receptor, discriminating between peptides of similar sequence [26]. There are many peptide-independent MHC-I receptors with important roles in immunity, including CD8, ILT2 and the Ly49 receptors (Fig. 1) [27–29]. These

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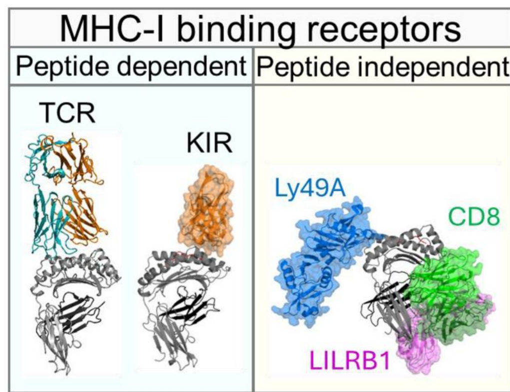

Figure 1. Peptide dependent and peptide-independent MHC-I binding receptors. Peptide-dependent receptors engage the MHC-I bound peptide directly (left). Peptide independent receptors engage MHC-I outside the peptide-binding groove (right).

receptors engage MHC-I molecules outside the peptide binding groove and are unable to sense peptide sequence directly. This broad structural distinction between peptide-dependent and peptide-independent receptors lends insight into the roles these receptor-ligand interactions play in immune responses. We deduce that the main function of peptide-independent receptors is to sense MHC expression level. It is possible that peptide-independent receptors can sense classes of peptides through allosteric changes in MHC-I structure, a mechanism that may be important for facilitating peptide exchange by molecules such as TAPASIN and HLA-DM [30, 31]. In contrast, peptide-dependent receptors engage the bound peptide directly, discriminating between peptides of different sequence. This is the quintessential role of the TCR, which uses six CDR loops to engage both MHC and peptide with exquisite specificity [14]. Further, we have known for many years that inhibitory KIR and CD94: NKG2A binding to MHC-I is peptide dependent but the functional relevance of this peptide "sensing" was unclear [32, 33]. More recently, activating members of these receptor families appear to engage HLA-I molecules with greater peptide specificity and may even detect "foreign" or pathogen derived peptides [20, 21, 23, 24]. In addition to peptide-dependent and peptide independent receptors, there are also reports of receptors that bind peptide empty HLA molecules (known as open conformers) [34–36], however these will not be covered here.

Inhibitory KIR

The KIR are named for the length of their cytoplasmic tails, which can be long (L) or short (S), and the number of extracellular Ig domains (2D) or (3D) [37] (Fig. 2). The Long cytoplasmic tails of inhibitory KIR contain two immunoreceptor tyrosine-based inhibitory motifs (ITIM), which are phosphorylated upon HLA-I binding, recruiting the phosphatase SHP-1, which inhibits adhesion to target cells and activating receptor signaling [38–40]. The human KIR genes are recently evolved, with equivalents only found in simian primates [41]. Ligands for inhibitory KIR are groups of HLA-I molecules with shared structural motifs, known as KIR epitopes [41]. KIR2DL1 binds HLA-C allotypes bearing the C2 epitope, while KIR2DL2 and KIR2DL3 (that are encoded by the same locus) bind C1 bearing HLA-C epitopes. KIR3DL1 binds a subset of HLA-A and HLA-B allotypes bearing the Bw4 serological epitope (Fig. 2A).

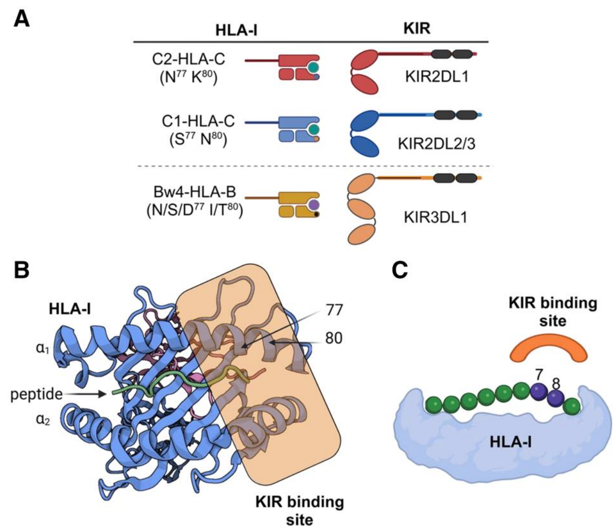

Figure 2. The KIR binding site on HLA-I. (A) KIR2DL1 binds to HLA-C allotypes with the C2 epitope. KIR2DL2/3 bind HLA-C allotypes with the C1 epitope. KIR3DL1 binds to HLA-B and HLA-A allotypes with Bw4 epitope. (B) The KIR binding site is located over the C-terminal end of the HLA-I bound peptide (green). (C) The KIR binding site incorporates peptide positions 7 and 8 (p7 & p8) of 9mer peptides.

Inhibitory KIR binding to HLA-I requires not only peptide stabilized HLA-I but is also dependent on peptide sequence [20, 32, 42–46]. Structural studies confirmed the KIR binding site to be over the C-terminal end of the peptide, covering peptide position 7 (p7) and p8 of 9mer peptides (Fig. 2B and C) [47–50]. Acidic residues at these positions appear universally unfavorable for KIR binding across multiple studies [20, 43–45, 51, 52]. For a summary of KIR binding studies that examined the contribution of HLA bound peptide is shown in Table 1. Soluble KIR molecules fused to human IgG constant regions (KIR-Fc) were developed as staining reagents, allowing many peptide sequences to be tested in one assay [20, 42–44, 53]. Functional experiments confirmed that strong binding of inhibitory KIR-Fc proteins to a given HLA-I peptide complex, inversely correlated with activation or lysis by KIR^+ NK cells [42–44]. Recent studies have focused in two main areas; (i) direct comparisons of peptide-selectivity by different inhibitory KIR and (ii) the KIR binding properties of pathogen derived peptides.

Directly comparing inhibitory KIR binding to C1 and C2 HLA-C using a large peptide library

Association studies implicate HLA-C binding KIR in multiple diseases including outcome of infections and reproductive success [54–56]. Understanding these associations requires a molecular understanding of how different KIR interact with HLA-I [57]. The dominant hypothesis for interpreting these associations is the strength of inhibition hypothesis, based on a hierarchy of KIR binding avidities for HLA-C [57]. Using KIR-Fc proteins, KIR2DL1 bound more strongly to $HLA-C^{2+}$ cells than KIR2DL2 or KIR2DL3 does to $HLA-C^{1+}$ cells [16]. However, direct affinity measurements by surface plasmon resonance showed that inhibitory KIR2DL1 and KIR2DL2/3 have similar binding strengths [20, 49, 52]. We reconciled these differences by proposing the peptide-selectivity model, which states that the dominant difference between KIRs is sensitivity to peptide sequence, not binding strength. For a full account of this model please see a recent review of this topic by us [58]. Using pairs of HLA-C allotypes that differ only by the C1/C2 epitope determining residues (positions

Table 1. Summary of studies that examined contribution of HLA-I bound peptide to KIR binding.

KIR	HLA-I	# peptides tested	Findings	References
3DL1 (p70)	B*27 : 05 (Bw4)	38	3DL1+ NK cells differentiate between HLA-B bound peptides of different sequence, including 'self' peptides. Performed substitutions along the peptide to identify p7 and p8 (of 9mers) to be critical for KIR binding and identified negatively charged residues as poor KIR binders.	[32, 45, 46]
2DL1	C*04 : 01 (C2)	17	2DL1 binding to HLA-C is peptide sequence dependent. Established 2DL1 binding site on HLA-I to be similar to 3DL1, with similar poor recognition of negatively charged residues at p7 and p8.	[42]
2DL2/3	C*03 : 04 (C1)	9	HLA-C eluted 'self' peptides confer a range of inhibitory capacity to 2DL2/3+ NK cells	[131]
2DL2	C*03 : 04 (C1)	8	Crystal structure of 2DL2 in complex with C1-HLA-C. Revealed direct contribution of peptide p7p8 to 2DL2 binding, with binding studies.	[49]
2DL1	C*04 : 01 (C2)	1	Crystal structure of 2DL1 in complex with C2-HLA-C. Revealed minimal contribution of peptide p7p8 to 2DL1 binding.	[47]
2DL1, 2DS1	C*04 : 01 (C2)	7	2DS1 binds weakly to HLA-C compared to 2DL1, but displays similar peptide selectivity	[17]
2DL2/3	C*01 : 02 (C1)	59	2DL2/3 binding to HLA-C is highly dependent on p7p8 sequence. Weak KIR binding peptides can 'antagonize' inhibition conferred by strong KIR binding peptides.	[44]
3DL1	B*57 : 01 (Bw4)	7	Crystal structure of 3DL1 with Bw4-HLA-B. Binding studies confirmed importance of peptide p8.	[50]
2DL2	C*01 : 02 (C1)	20	2DL2 binds HLA-C presenting HIV $Gag_{209-218}$. HIV sequence variation at p7 of this 10mer epitope impacted 2DL2 binding. Showed that viruses can encode KIR binding peptides.	[69]
2DL2/3	C*03 : 04 (C1)	222	Screened HIV proteome overlapping 10mer peptides for HLA-C*03 : 04 binding peptides and HIV derived 2DL2/3 binding peptides. HIV sequence variation in one Gag epitope influenced 2DL3 binding.	[67, 68]
2DL2/3	C*03 : 04 (C1)	10	Identified HCV encoded HLA-C*03 : 04 restricted 2DL2/3 binding peptides	[66]
2DL1, 2DL2/3	C*05 : 01 (C2), C*08 : 02 (C1)	28	Demonstrated that 2DL2/3 binding to C1-HLA-C is more dependent on peptide sequence than 2DL1 binding to C2-HLA-C.	[43]
3DL1	B*57 : 01 (Bw4) B*27 : 05 (Bw4)	9 5	3DL1 binding to Bw4-HLA-I is influenced by peptide p8 sequence and 3DL1 polymorphism at residue 283.	[132]
3DS1	B*57 : 01 (Bw4)	7	3DS1 binding to HLA-B*57 : 01 is dependent on peptide p8 sequence. Identified two HIV derived 3DS1 binding peptides.	[133]
2DS2	C*01 : 02 (C1) C*03 : 04 (C1)	11 3	Identified 2DS2 binding peptides that stimulate 2DS2+ NK cells. 2DS2 epitopes were identified in *Flaviviruses* including Zika/Dengue, Yellow Fever and West Nile.	[24]
2DS4	C*05 : 01 (C2)	76	Identified 2DS4 binding peptides that stimulate 2DS4+ NK cells. 2DS4 epitopes include an endogenous 'self' peptides and partially conserved bacterial peptides derived from Recombinase A (RecA).	[21]
2DL1, 2DL2/3, 2DS1, 2DS4	C*05 : 01 (C2), C*08 : 02 (C1)	365	Compared KIR binding to a library of peptides comprised of every amino acid combination at p7 and p8. Demonstrated that KIR2DL1 is largely insensitive to peptide-sequence, while KIR2DL2/3, KIR2DS1 and KIR2DS4 are more peptide-specific.	[20]
	C*16 : 01 (C1), C16:02 (C2)	87	Confirmed conclusions obtained studying C*05 : 01 and C*08 : 02. Comparing p7p8 motifs suggested some shared KIR binding modes.	
2DS2	C*01 : 02 (C1)	1	Identification of 2DS2 binding peptides derived from XPO1 and upregulated in tumors.	[86]

77 and 80), we studied KIR binding to HLA-C in the presence of the same peptides in the context of either C1 or C2 defining residues [20, 43]. We screened a library of 361 peptides containing every amino acid residue at p7 and p8 for binding to inhibitory KIR2DL1/2/3 and activating KIR2DS1/4 (described below). We found that KIR2DL1 bound to C2-HLA-C in the presence of approximately 60% of peptides [20], while KIR2DL2 and KIR2DL3 bound to C1-HLA-C in the presence of approximately 10% of peptides. These data support a model whereby sensitivity to peptide sequence, not binding strength, is the differentiating feature between KIRs. The differences between KIR2DL1-C2 and KIR2DL2/3-C1 may contribute to our understanding of diseases associated with HLA-I and KIR [55–57, 59]. Work by another group that non-KIR binding peptides could "antagonize" the function of strong KIR binding peptides, reversing the inhibitory signals from KIR2DL2/3 [44]. In this experimental setting, they observed KIR clustering however the receptors failed to transduce the signal beyond recruiting SHP-I [60, 61]. These data are hard to reconcile with the fact that as many as 90% of HLA-C bound peptides are incompatible with KIR2DL2/3 binding [20]. However, it does

support further investigations into how changes in the immunopeptidome on the function of KIR^{+} NK cells, specifically in response to infection.

Pathogen derived peptides

Disease association studies link specific KIR-HLA-I combinations with resolution of viral infections [55, 62–64]. Known pathogen derived peptides that bind KIR are summarized in Table 2. Hypothetically, it would be advantageous to a virus to maintain strong inhibitory KIR binding by encoding HLA-I bound peptides that are strong KIR ligands. This is most likely to impact the more peptide-selective inhibitory receptors such as KIR2DL2/3 and KIR3DL1. The combination of KIR3DL1 and its ligand HLA-Bw4 are associated with delayed progression to AIDS in HIV infected individuals [62]. A study of known HIV T cell epitopes bound to HLA-Bw4 allotypes identified both poor and strong KIR3DL1 binders [65]. Furthermore, HIV escape mutations known to eliminate T cell recognition also abrogated KIR3DL1 binding [65], suggesting that these same mutations may lead to decreased inhibition of KIR3DL^{1+} NK cells engaging HIV infected cells that bear these escape variants. For the HLA-C binding KIR, multiple studies identified strong KIR2DL2/3 binding peptides derived from viruses including those from HIV and HCV [66–69]. However, the HLA-C immunopeptidome contains hundreds to thousands of different sequences [70, 71] and it is not clear how the introduction of one or several pathogen derived peptides would impact KIR binding overall. Furthermore, upon viral infection the largest changes to the immunopeptidome appear to be from endogenously derived peptides and not viral sequences, and the KIR binding properties of these sequences are unknown [72, 73]. One study found that the most abundant “self” peptide presented on uninfected CD^{4+} T cells as a strong KIR2DL3 binder, while the most abundant peptide on HIV-infected CD^{4+} T cells was a weak KIR2DL3 binder [73]. These data suggest that not only peptide sequence, but peptide abundance may be key regulators of KIR binding and NK cell responses to viral infections.

Activating KIR

The activating KIR have short cytoplasmic tails and co-ordinate with ITAM containing signaling adaptors to propagate signaling upon receptor engagement [74, 75]. Many activating KIR exhibit high sequence homology with inhibitory KIR, however their ligands for were difficult to define, with some research suggesting that activating KIR have lost HLA-I binding [19]. It is now clear that many activating KIR do bind HLA-I, but do so in a far more peptide specific manner than inhibitory KIR [20, 21, 24]. KIR2DS1 displays binding to HLA-C^{2+} cells, but with weaker avidity than KIR2DL1 [17, 19]. In contrast, early data showed that KIR2DS2 and KIR2DS4 display no or very weak binding to HLA-I^{+} cells, with some reports of HLA independent ligands [16, 18, 76, 77]. However, more recent experiments identified specific peptides presented by HLA-C that confer activation of KIR2DS1^{+}, KIR2DS2^{+} and KIR2DS4^{+} NK cells and include peptides conserved in pathogens [21, 24]. Direct measurements of affinity demonstrated that activating KIR are not weak receptors but of similar affinity to inhibitory KIR [20].

KIR2DS4

KIR2DS4 was previously shown to bind a selection of HLA-I allotypes including HLA-C*04 : 01 [18], HLA-A*11 : 02, HLA-C*16 : 01 and HLA-C*05 : 01 [78]. However, there was no evidence that the interaction with HLA-C was functional and the contribution of peptide sequence to KIR2DS4 binding was unknown [18, 78]. KIR2DS4 has a direct orthologue in Chimpanzee, which also bound HLA-A*11 : 02 and HLA-C*16 : 01 but not HLA-C*05 : 01 [78]. Like the inhibitory KIR, genetic association studies link carrying KIR2DS4 with diseases including protection against pre-eclampsia and glioblastoma, but also with higher viral loads and an increased risk of developing AIDS in HIV infection [79–82]. In an initial study, we identified a modified “self” peptide, P2-AY (IIDKSGAYV), which bound KIR2DS4 when presented by HLA-C*05 : 01 [21]. By testing other amino acids with aromatic side chains, we identified P2-AW (IIDKSGAWV) with Trp at p8 as an

Table 2. Pathogen derived peptides that bind KIR.

KIR	HLA-I	Pathogen	Protein	Epitope	References
3DL1	B*57 : 01 (Bw4)	HIV	$Gag_{240\text{-}249}$	TSTLQEQIGW	[65, 133]
			$Gag_{162\text{-}172}$	KAFSPEVIPMF	[65]
			$Gag_{147\text{-}158}$	LTVQVARVY	
	A*24 : 02 (Bw4)		$Gag_{162\text{-}172}$	RDYVDRFFKTL	[65]
			$Gag_{28\text{-}36}$	KYRLKHIVW	
			$Nef_{131\text{-}141}$	RYPLTFGW	[133]
2DL2/3	C*01 : 02 (C1)	HIV	$Gag_{209\text{-}218}$	AAEWDRLHPV	[69]
2DL2/3	C*03 : 04 (C1)	HIV	$Gag_{144\text{-}152}$	HQAISPRTL	[68]
			$Gag_{163\text{-}171}$	AFSPEVIPM	
			$Gag_{295\text{-}304}$	DYVDRFFKTL	
2DL2/3	C*03 : 04 (C1)	HCV	$Core_{136\text{-}144}$	YIPLVGAPL	[66]
2DS2	C*01 : 02 (C1)	HCV	NS3 $helicase_{1258\text{-}1266}$	LNPSVAATL	[24]
		Zika/Dengue	NS3 $helicase_{257\text{-}266}$	IVDLMCHATF	
		Yellow Fever		VIDAMCHATL	
		West Nile/JEV		IVDVMCHATL	
3DS1	B*57 : 01 (Bw4)	HIV	$Pol_{839\text{-}847}$	AAVKAACWW	[133]
			$Nef_{82\text{-}90}$	KAAFDLSFF	
2DS4	C*05 : 01 (C2)	*Campylobacter jejuni*	Recombinase $A_{283\text{-}291}$ (RecA)	IVDKSGAWF	[21]
		Chlamydia trachomatic		IIDKKGSWF	
		Brucella abortus		VVEKSGAWF	
		Helicobacter pylori		IVDKSGAWL	
2DS4	C*05 : 01 (C2)	*Escherichia coli*	Type F conjugate transfer system pilin chaperone (TraQ)	RLDIRGMWV	[20]
	C*08 : 02 (C1)	*Klebsiella pneumonia*			
		Salmonella enterica			
		Shigella sonnei			
		Acinetobacter baumannii			

even stronger KIR2DS4 ligand [21]. The importance of p8 Trp for KIR2DS4 ligands was validated in a large screen of every amino acid combination at p7p8, where only peptides containing p8 Trp conferred strong KIR2DS4 binding [20]. However, only specific combinations of p7 and p6 with p8 Trp were sufficient for KIR2DS4 binding [20]. Reminiscent of T cell activation, KIR2DS4$^+$ NK cells were specifically activated by HLA-C*05 : 01$^+$ target cells when presenting such peptides [20, 21].

The biological role of peptide recognition by KIR2DS4$^+$ NK cells is not clear. We first searched HLA-C*05 : 01 immunopeptidomes for peptides with p8 Trp and identified *bone fide* "self" peptides that were KIR2DS4 ligands [21]. As such, immune tolerance by KIR2DS4$^+$ NK cells is likely determined by the number of cell surface peptides, and not by strict "self/non-self" discrimination. Next, we conducted an alignment search for sequences similar to P2-AW and identified a KIR2DS4 epitope derived from residues 283–291 of the recombinase A (RecA) protein from the bacterium *Helicobacter* fenneliae, differing only by a single amino acid [21]. RecA is a highly conserved bacterial protein responsible for homologous DNA repair [83]. In an alignment of 63 $RecA_{283-291}$ sequences, 61/63 carried the Trp at peptide position 8 and of those that stabilized HLA-C*05 : 01 well, we identified KIR2DS4 epitopes carried by the human pathogens Campylobacter jejuni, Brucella abortus, Chlamydia trachomatis and H. pylori [21]. Further, we searched the UNIPROT protein database using a combined KIR2DS4 and HLA-C*05 : 01 binding motif and identified hundreds of predicted peptides from bacterial genomes [20]. These data suggest that KIR2DS4 evolved to play a role in innate immune defense to bacteria [84], however evidence from a role of KIR2DS4$^+$ NK cells in control of bacterial infection is still lacking.

KIR2DS2

Unlike human KIR2DS2, chimpanzee activating KIR display binding to C1-HLA-C, leading to the conclusion that perhaps KIR2DS2 had evolved to lose HLA-I binding [16, 19]. However, more recent evidence demonstrated KIR2DS2 can bind HLA-C in a peptide-specific manner [24]. Using KIR2DS2 tetramers, Naiyers *et al.*, tested p7p8 variants of a known KIR2DL2 binding peptide bound by HLA-C*01 : 02. They identified the p7p8 combination Ala and Thr (AT) as conferring KIR2DS2 binding. They next identified carrying *KIR2DS2* and *C1-HLA-C* with protection against chronic HCV infection and searched for potential KIR2DS2 binding peptides in viral genomes containing the "AT" motif. A conserved KIR2DS2 binding peptide shared by the NSP3 of Flaviviruses was identified in multiple viral species including Ebola, Zika, West Nile and Hepatitis C virus. In a follow-up study, Rettman and colleagues designed DNA vaccine constructs that included the two validated peptides from HCV and Zika/Dengue identified in Naiyer *et al.*'s research. Their goal was to develop a peptide-based vaccine strategy targeting KIR. They discovered that immunizing KIR transgenic mice with their DNA vaccine could activate KIR2DS2$^+$ NK cells, which provided protection in a mouse tumor model expressing HLA-C*01 : 02 [85]. Blunt *et al.* also validated another functional "AT" motif-containing peptide derived from XPO1, a nuclear exporter upregulated in many cancer types [86]. A structural analysis on KIR2DS2-HLA-C*01 : 02 further confirmed the importance of the "AT" motif. They found that p8 Thr is the most important for binding to KIR2DS2, as it forms a hydrogen bound with Gln at position 71 of KIR2DS2 [87]. These studies highlight the potential for KIR2DS2$^+$ NK cells to contribute to immunity to both tumours and viruses via peptide-specific recognition of HLA-C.

KIR2DS1

Of the activating KIR2D, KIR2DS1 displays binding to HLA-C$^+$ cells, but with weaker avidity than KIR2DL1, its highly related inhibitory receptor [17, 19]. One study of seven peptides bound to HLA-C*04 : 01 (C2) found that KIR2DS1 and KIR2DL1 displayed a similar preference for peptide sequence, but KIR2DS1 was a weaker receptor [17]. However, using our large peptide screen with every combination of amino acids at p7p8, we showed that KIR2DS1 is highly peptide-specific receptor and categorically showed using biacore that KIR2DS1 is not a weak receptor [20]. Supporting this idea, KIR2DS1$^+$ reporter cells and NK cell clones responded to HLA-C*06 : 02$^+$ (C2) cells loaded with a specific peptide [88]. Peptide-specific recognition by KIR2DS1$^+$ NK cells may play a role in immune defense against human cytomegalovirus (HCMV) infection [89]. While the specific viral peptides have not been identified, KIR2DS1$^+$ reporter and primary NK cells responded to HCMV infected fibroblasts [89]. Intriguingly, only certain HCMV strains induced KIR2DS1$^+$ cell activation pointing to sequence variation and thus differential HLA-C bound peptides being presented and detected by KIR2DS1$^+$ NK cells [89]. Pregnancies where the mother carries KIR2DS1 is associated with protection from pre-eclampsia and higher birth weight [90, 91]. Also, donor grafts that carry KIR2DS1 is associated prevention of leukemia relapse [92]. Our data suggest that these associations will rely on the detection of specific peptides by KIR2DS1$^+$ NK cells, however these peptide sequences are undetermined.

CD94: NKG2A

The CD94: NKG2A heterodimer (NKG2A) is expressed on approximately 30–60% of NK cells in both humans and mice [93–95]. Its ligand is the non-classical MHC-I molecule HLA-E, Qa-1 in mice, and carried by all individuals. HLA-E is largely monomorphic and its primary function is to present peptides derived from the leader sequences of other HLA-I molecules [96]. The peptide binding groove of HLA-E is highly restrictive and specialized for this role. By binding peptides derived from nascent polypeptides of other HLA-I molecules, cell surface HLA-E serves as a general mechanism for indicating HLA-I expression level [95, 97]. If a pathogen targets classical HLA-I for downregulation, the number of HLA-E binding signal sequences will decrease leading to decreased HLA-E expression, allowing NKG2A$^+$ NK cells to respond via missing self.A summary of NKG2A/C binding peptides is shown in Table 3.

While the peptide binding repertoire of HLA-E is highly restricted, NKG2A still discriminates between peptides of different

Table 3. NKG2A/C binding peptides.

Receptor	Peptide	Source protein	Species	References
NKG2A/C	VMAPRTLFL	$HLA\text{-}G_{3\text{-}11}$	Human	[33, 95, 97, 107]
	VMAPRTLLL	$HLA\text{-}A_{3\text{-}11}$		[97]
	VMPPRTLLL	$HLA\text{-}A_{3\text{-}11}$		
	VMAPRTLIL	$HLA\text{-}C_{3\text{-}11}$		
NKG2A/C	VMAPRTLFL	$UL40_{15\text{-}23}$	HCMV	[23, 101]
	VMAPRTLIL			
	VMAPRTLVL			
NKG2A/C	RMPPRSVLL	$INTS1_{260\text{-}268}$	Human	[106]
	TLPERTLYL	$ECEL1_{269\text{-}277}$		
	VMPGRTLCF	$TACR3_{226\text{-}234}$		
	ILTDRSLWL	$FBXO41_{670\text{-}678}$		
	NMPARTVLF	$MTREX_{490\text{-}498}$		
	VLPHRTQFL	$UL120_{72\text{-}80}$	HCMV	
NKG2C	VNPGRSLFL	$BFAR_{263\text{-}271}$	Human	

sequence [33, 97]. A recent comprehensive analysis of HLA-I signal sequences identified a range of HLA-E and CD94: NKG2A interactions [97]. The HLA-G_{3-11} signal sequence (VMAPRTLFL) conferred strongest binding to HLA-E and NKG2A, while other leader sequences such as “6B” (VMAPRTLVL) and “3C” (VMAPRALLL) conferred much weaker NKG2A binding despite strong HLA-E stabilization [97]. The structure of CD94: NKG2A in complex with HLA-E presenting the HLA-G leader sequence (VMAPRTLFL) revealed that CD94 subunit made 11 peptide contacts, while NKG2A made only one [98]. Five of these contacts were with p8 Phe, suggesting that differences at p8 in signal peptides “6B” and “3C”, Val and Leu respectively, can detected by CD94: NKG2A. The “3C” peptide also contains p5 Ala that likely eliminates 3 contacts made by p5 Thr, perhaps contributing to “3C” being a poorer CD94: NKG2A ligand than “6B” [97]. Thus, CD94: NKG2A discriminates between HLA-E bound peptides [99].

Direct pathogen sensing

There are multiple cases of pathogen derived peptides binding to HLA-E despite its limited peptide repertoire [100–102]. The clearest case is from HCMV that encodes an HLA-E binding peptide from the UL40 protein [101]. This sequence is identical to HLA-I signal sequences and confers strong binding to both HLA-E and NKG2A, serving as an immunoevasion strategy for HCMV by ensuring that HLA-E expression is maintained and NKG2A$^+$ NK cells are inhibited. This may also allow HCMV to evade adaptive immunity through downregulation of classical HLA-I, which would typically lead to a decrease in HLA-E surface expression. Other pathogens encoding HLA-E binding peptides include *Mycobacteria tuberculosis* (*M.tb*) and SARS-CoV2 [100, 102]. Multiple studies have identified HLA-E restricted T cells to epitopes derived from *M. tb,* however whether these peptides can bind NKG2A is unknown [102, 103]. Much like HCMV UL40, SARS-CoV2 encodes a peptide that strongly binds HLA-E (VMPLSAPTL), derived from Nsp$13_{232-240}$ [100]. However, this peptide confers no binding to NKG2A, again demonstrating the peptide discrimination capacity of NKG2A [100]. This peptide is likely to relieve inhibition of NKG2A$^+$ NK cells and most likely advantageous to host innate immunity to SARS-CoV2. Recent evidence showed that a p2 variant (M2I) of this epitope (VIPLSAPTL) derived from SARS-CoV2 Omicron BQ.1 sub-variant has reduced HLA-E binding [104]. The authors hypothesize that by reducing HLA-E binding of this epitope will restore presentation of VL9 “self” peptides, thus restoring NKG2A mediated inhibition of NK cells and may improve viral fitness [104].

CD94: NKG2C

NKG2C, like NKG2A pairs with CD94, but is an activating receptor and signals through DAP12 [74]. NKG2C shares x% homology with NKG2A in their ectodomains and binds HLA-E with a general lower affinity than NKG2A [95, 97, 105]. NKG2C displays very similar peptide recognition properties to NKG2A, with affinities approximately 2–3 fold lower [97, 106]. A high-throughput screen of NKG2A and NKG2C peptide specificities revealed remarkably similar binding motifs and identified novel human and viral derived NKG2A/C binding peptides [106]. Like NKG2A, the best characterized NKG2C ligand is HLA-E presenting the HLA-G leader sequence, which induced functional responses in NKG2C$^+$ NK cells [23, 105–107]. Under physiological conditions, HLA-G is most highly expressed on extra-villous trophoblasts (EVT) at the maternal fetal interface with the potential to interact with NKG2C$^+$ uNK cells [5, 6]. Activated uNK cells are thought to protect against pregnancy disorders, such as pre-eclampsia and fetal growth restriction via promoting EVT invasion, which is crucial for ensuring adequate nutrient transfer across the placenta [5, 6]. It is possible that recognition of the HLA-G leader peptide contributes to activation of uNK cells and successful pregnancy as has been suggested for activating KIR [6, 79, 90].

Peptide-specific recognition of HLA-E may also contribute to immune defense against HCMV [23]. In the mouse, the activating receptor Ly49H specifically recognizes the MHC-I-like murine CMV protein m157 expressed on MCMV infected cells [108]. Ly49H$^+$ NK cells specifically expand *in vivo* during MCMV infection forming a pool of memory NK cells that protect against future MCMV challenge [109]. While not entirely analogous, NKG2C$^+$ NK cell populations dominate the peripheral blood of humans exposed to HCMV [110]. These “adaptive” NK cells are the product of clonal expansions and are characterized by a unique transcriptomic, epigenetic signature and functional properties [111, 112]. These adaptive populations are defined by CD57 and NKG2C surface expression as well as loss of FcRγ and transcription factor PZLF [111]. There is also evidence for NK cell clonal-like expansions in HCMV infected individuals that are NKG2C$^-$ activating KIR$^+$ [113]. Primary HCMV isolates displayed considerable sequence variation in UL40, which modulated recognition of the HLA-E binding UL40 peptide by NKG2C$^+$, but not NKG2A$^+$ NK cells [23]. A sequence analysis of over 200 primary HCMV isolates revealed 1.4% of UL40 sequences contain F at p8, and conferred strong stimulation of NKG2C$^+$ NK cells [23]. The most common UL40 variant contained p8 I, followed by L and V, and conferred weaker activation of NKG2C$^+$ NK cells, while other variants conferred no activation [23]. It is likely that detection of UL40 peptides by NKG2C$^+$ NK cells contributes to the formation of this “adaptive” NK cell population [114], however there is evidence for adaptive NK cells in NKG2C negative individuals [115], indicating the contribution of other receptors to the formation of “adaptive” NK cells. Another HCMV peptide derived from UL120 (VLPHRTQFL) also stimulated NKG2C$^+$ NK cells, but only when derived from the Merline strain [106]. Thus, NKG2C can discriminate between HLA-E bound HCMV derived peptides and this contributes to innate immunity to HCMV.

NKp44: a peptide-specific receptor for HLA-II?

It is well established that MHC-I molecules play a critical role in regulating NK cell function but the role of MHC-II molecules is less clear [116]. NKp44 is a natural cytotoxicity receptor (NCR) with multiple reported ligands [117]. In 2019, Niehrs *et al.* reported that NKp44 could also bind a subset of HLA-DP allotypes [22, 118, 119]. They conducted a screen of soluble NK cell receptors for binding HLA-II allotypes and found that NKp44, but not NKp30 or NKp46 could bind certain HLA-DP allotypes and could discriminate between different HLA-DP bound peptides [22]. NKp44 is non-polymorphic and carried by all individuals, however not everyone carries HLA-DP allotypes that are NKp44 ligands [116]. As such, an interaction with NKp44$^+$ NK cells could explain multiple disease associations implicating certain HLA-DP alleles including ulcerative colitis and primary sclerosing cholangitis [22, 118, 119]. Future work should also focus on the molecular and structural basis for peptide discrimination by NKp44.

Evolutionary models for innate peptide-specificity

Receptors with broad peptide recognition, like inhibitory KIR2DL1, are best suited to sensing the pMHC surface levels and are likely highly tolerant of changes in MHC bound peptide-repertoire as may occur during infection. More peptide-selective receptors and PSNKRs are better suited to detect subtle changes in the peptide repertoire or specific peptides with highly conserved motifs. The challenge for peptide-specific activating receptors is to achieve sufficient specificity while remaining "useful" and to not promote a large risk of autoimmunity. While harmful TCRs are largely pruned by thymic negative selection [120], the specificities of germline-encoded receptors like KIR are subject to evolutionary forces of positive, negative and balancing selection [41]. TCRs "see" MHC bound peptides, a sequence of amino acids, however in many cases their immunological purpose can be reasonably ascribed based on the source of their peptide ligands. Conversely, the biological role of PSNKRs is not so apparent as the disease contexts that drove their evolution may be very different to today.

The specificity of PSNKRs so far defined are peptides conserved across a broad range of viruses and bacteria [21, 24], therefore enabling the receptors to efficiently recognize highly variable pathogens. One model for PSNKR evolution is based on that of NKG2A and NKG2C. HCMV has evolved numerous immunoevasion strategies including presentation of a UL40 peptide bound to HLA-E that engages NKG2A and thus inhibits NK cell activation. This allows other HCMV proteins such as US11 to downregulate classical HLA-I molecules to evade T cell recognition [121]. In response, the host may have evolved an activating receptor that can detect this high HLA-E expression through gene duplication. The KIR evolved recently and appear highly specialized in different primate species. The "arms race" model seems suited to the activating KIR, which evolved from inhibitory receptors via gene duplication [122]. KIR2DS1 and KIR2DS2 have inhibitory counterparts that evolved in the human lineage [122] and a conserved viral epitope has been identified as a ligand for KIR2DS2 [24]. One argument against this model is that pathogens evolve more rapidly than their hosts. Indeed, six MCMV passages through mice carrying Ly49H is sufficient to generate m157 variants that eliminate this interaction [123]. However, its clear that HCMV has made a significant impact on polymorphic human immune genes [121] and its likely other pathogens have impacted the evolution of PSNKRs.

For the KIR locus, another evolutionary driver appears to be reproductive success, where HLA-C binding activating KIR are associated with protection from pregnancy disorders and higher birth weight [79, 90, 91, 124]. The C2-HLA-C epitope evolved recently and is only found in humans, gorillas, bonobos and chimpanzee [41, 125]. While we know that EVT express HLA-C, we do not know the peptides presented on these cells, which presumably present numerous peptide ligands for activating KIR. The selective advantage of activating receptors that promote successful pregnancy and higher birth weight may be counteracted by a greater risk of autoimmunity and too high a birth-weight [90, 122, 126–128]. By further defining PSNKR ligands from different proteomes, we will begin to understand what roles they can and cannot play in immunity.

Future directions

We know a tremendous amount about how pMHC are detected by TCRs, but comparatively very little about other highly peptide-sequence dependent receptors detect MHC bound peptides. Current studies rarely consider the proposition that MHC bound peptide may impact NK cell receptors and NK cell activation. Key limitations are the lack of high-throughput methods to determine PSNKR specificities however, a viable strategy would include adapting existing methods to determine TCR specificity [129]. Combined with a molecular and structural understanding of this innate peptide-specificity will facilitate building computational tools to predict PSNKR ligands from sequence. These tools should be used to scan immunopeptidomes and proteomes to identify *bone fide* PSNKR ligands. The selective advantage of peptide-specific activating receptors appears to be innate detection of broad classes of pathogens, somewhat analogous to PRR. However, the evolutionary advantage of peptide-selective or peptide-specific inhibitory receptors is less clear. For inhibitory KIR, peptide selectivity appears to differentiate the binding between very similar KIR-HLA-I interactions [20, 43, 58, 130]. The advantage of this selectivity maybe to respond to changes in peptide repertoire that may occur during infection or transformation, however studies are yet to conclusively demonstrate this. NKG2A also has the potential to detect changes in peptide repertoire, especially where infections specifically target the HLA-E antigen presentation pathway such as HCMV [101].

Conclusion

Peptide-specificity is a biochemical property of receptors that engage MHC molecules. Receptors that tolerate more peptide sequence diversity are less peptide-specific, while highly peptide-specific receptors only engage MHC when presenting a limited number of peptides. Its clear that innate, germline-encoded peptide-specific receptors exist, with many similarities to TCRs. Ongoing research into the specificities of these innate receptors will hopefully shed light on the biological purpose of these PSNKRs. Future research will aim to identify shared and distinct features of peptide recognition by TCRs and PSNKRs, providing further distinction of innate and adaptive immune systems, respectively.

Author contributions

Malcolm Sim (Conceptualization [equal], Writing—original draft [lead], Writing—review & editing [equal]), Beining Li (Writing—original draft [supporting], Writing—review & editing [supporting]), and Eric O. Long (Conceptualization [equal], Resources [lead], Writing—review & editing [equal])

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