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Structural insights into activation of antiviral NK cell responses

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Summary

Natural killer (NK) cells are key components of innate immune responses, providing surveillance against cells undergoing tumorigenesis or infection, by viruses or internal pathogens. NK cells can directly eliminate compromised cells and regulate downstream responses of the innate and acquired immune systems through the release of immune modulators (cytokines, interferons). The importance of the role NK cells play in immune defense was demonstrated originally in herpes viral infections, usually mild or localized, which become severe and life-threatening in NK-deficient patients (1). NK cell effector functions are governed by balancing opposing signals from a diverse array of activating and inhibitory receptors. Many NK receptors occur in paired activating and inhibitory isoforms and recognize major histocompatibility complex (MHC) class I proteins with varying degrees of peptide specificity. Structural studies have made considerable inroads into understanding the molecular mechanisms employed to broadly recognize multiple MHC ligands or specific pathogen-associated antigens and the strategies employed by viruses to thwart these defenses. While many details of NK development, signaling, and integration remain mysterious, it is clear that NK receptors are key components of a system exquisitely tuned to sense any dysregulation in MHC class I expression, or the expression of certain viral antigens, resulting in the elimination of affected cells.

Keywords

natural killer cell; structural molecular immunology; immunoreceptors; viral evasion; KIR; LILR

Introduction

Natural killer cells

Natural killer (NK) cells play key roles in combating infections with many viruses, including human immunodeficiency virus (HIV), influenza virus (IV), hepatitis viruses, poxviruses, and herpesviruses, by (i) directly lysing infected cells and (ii) by promoting antiviral adaptive immune responses through interactions with dendritic cells (DCs) and through the release of cytokines (2,3). NK cells are large granular lymphocytes with cytotoxic activity. In humans, NK cells can be divided into subsets, based on expression levels of two cell-surface markers, CD56 and CD16 (CD56^{dim}CD16⁺ versus CD56^{bright}CD16⁻ NK cells), which differ in their effector functions and homing properties (reviewed in 4). Around 90% of NK cells found in the peripheral blood and spleen are CD56^{dim}CD16⁺ and likely develop from CD56^{bright}CD16⁻ precursors (5). CD56^{dim}CD16⁺ NK cells are cytotoxic and express high levels of perforin and the low affinity Fc γ receptor CD16. CD56^{bright}CD16⁻ NK cells, in contrast, predominate in the secondary lymphoid tissues (lymph nodes and mucosa-associated lymphoid tissues) and are copious producers of cytokines but are only weakly cytotoxic.

NK cell antiviral functions are governed by the integration of potentially opposing signals received through a repertoire of germline-encoded, activating or inhibitory, cell surface receptors that recognize and respond to the presence or absence of ligands on virally infected cells and tumor cells (Table 1). With the exception of CD16, which recognizes the Fc portion of an antibody and mediates antibody-dependent cell-mediated cytotoxicity (ADCC), full activation of NK cell effector functions requires stimulation through at least two receptors or through one receptor plus cytokine stimulation (6,7). Activating and inhibitory receptors are thought to signal based on the missing-self and non-self/alterd-self hypothesis. In the missing-self hypothesis, downregulation of human leukocyte antigen (HLA) proteins, encoded at three loci within the major histocompatibility complex (MHC), HLA-A, HLA-B, and HLA-C, constituting the 'classical' MHC class I proteins, or the 'nonclassical' MHC class I protein HLA-E, leads to the loss of inhibitory receptor interactions and subsequent disinhibition of cytotoxic activity (8–11). MHC class I proteins are heterodimers composed of the membrane-spanning and highly polymorphic heavy chain (α -chain) associated with the non-polymorphic light-chain β_2 -microglobulin (β_2m) (Fig. 1). They present peptide fragments, generally derived from endogenously expressed proteins, within a groove formed by the $\alpha 1$ and $\alpha 2$ sequence domains of the heavy chain (together forming the $\alpha 1/\alpha 2$ 'platform' structural domain); this allows the adaptive immune system to survey the proteome of any given cell through interactions with T-cell receptors (TCRs). Viruses and tumors will downregulate class I proteins to evade T-cell-mediated responses; this action, however, leaves them susceptible to NK cell attack through missing-self recognition. In the non-self or altered-self hypothesis, activating receptors recognize MHC class I proteins presenting viral or stress associated peptides or directly detect intact, virally encoded proteins (12–16).

A historical perspective

We are fortunate to be able to take for granted our understanding of the molecular underpinnings of MHC restriction and antigen presentation to TCRs. However, the process of achieving that understanding illustrates the power and efficiency of structural biology to inform us about the details of protein function recalcitrant to alternate experimental approaches. One of the authors (RKS) is able to provide a perspective, as a side-line observer to that process (a junior graduate student in the mid-1980s), that is particularly *apropos*. The first MHC class I crystal structure (of HLA-A2) was determined by Pamela Bjorkman in 1987 in Don Wiley's group at Harvard University (17, 18). The contemporaneous first-year, graduate-level immunology course at Harvard was taught by the eminent John Kimball using his own, recently published textbook (19). The class covered the consensus understanding of antigen presentation at that time (reviewed in 20), which included receptor, receptor-and-a-half, and two receptor models, with antigen, in an as yet undefined form, somehow associated with 'restricting elements' (HLA proteins) for presentation at the cell surface. Models of the process proposed that restricting elements bound intact or minimally processed antigens, raising the very significant question of how the limited repertoire of HLA proteins in a given host could bind to the huge array of potential antigens in way consistent with the rules of protein recognition, even as understood at that time (the suggestion that 'antigen fragmentation' may be both necessary and sufficient for presentation by restricting elements was first forwarded in 1983) (21). The conflict between proposed models of antigen presentation to TCRs and established rules of protein recognition was palpable, as was the tenuous nature of communication between the disparate disciplines. The initial details of the HLA-A2 structure, including the now famous observation that '[a] large groove between the α -helices provides a binding site for processed foreign antigens', revealed the logical and elegant mechanism of peptide presentation that we now understand and appreciate. The HLA-A2 structure (22) remains perhaps one of if not the best example of how a single, timely protein structure can not only

resolve a scientific conundrum but rewrite entire fields. The impact of the HLA-A2 structure also cemented the ties between the structural and immunological communities that have since resulted in many significant advances. In this review, we endeavor to identify systems where structural approaches have been particularly fruitful and questions that may yet also be resolvable by the application of modern structural molecular immunology, particularly in regard to ligand recognition by NK receptors and the strategies employed by viruses to evade them.

MHC class I-specific receptors

TCRs bind complexes of polymorphic MHC class I proteins and antigenic peptides with the capability of recognizing even small changes in the peptide or HLA protein sequences. The huge array of specificities needed is generated by blending combinatorial gene segment rearrangement and recombination (to generate a large repertoire of unique receptors) with plastic-binding sites engineered to sample a wide range of conformers [to incorporate functional polyspecificity (23) into each receptor]. Multiple lines of evidence also argue that TCRs and MHC class I proteins have co-evolved as interaction partners (24–28). However, NK cell receptors are germline encoded and, with few exceptions, display conventional rigidities. Therefore, fundamental questions to be answered by structural studies of NK receptor interactions with MHC class I proteins include understanding (i) whether and how these interactions can encompass HLA class I protein diversity without the mechanisms enabling TCR polyspecificity and (ii) how these interactions *in toto* might restrict or affect MHC class I evolution in regards to its role as a ligand for a panel of divergent receptors on various cell types.

The killer-cell immunoglobulin-like (KIR) family of receptors

The highly polymorphic KIR receptor family is encoded on chromosome 19q13.4 within the leukocyte receptor complex (LRC) and is expressed on NK and T cells. Members of the KIR family are Type I transmembrane glycoproteins that can have ectodomains comprising two (KIR2D) or three (KIR3D) immunoglobulin (Ig)-like domains (named D0, D1, and D2), can deliver inhibitory or activating signals upon ligand engagement, and share >90% sequence identity in their extracellular domains (29). Inhibitory KIRs (designated by an 'L') have long cytoplasmic tails that contain two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and therefore have the capacity to inhibit cellular activity. Activating KIRs (designated by an 'S') have short cytoplasmic tails that contain a positively charged residue in the transmembrane (TM) region. This residue (Arg or Lys) interacts with a complementary charged residue in the TM of the immunoreceptor tyrosine-based activation motif (ITAM)-containing adapter molecule DAP12 to deliver activating signals (30).

Inhibitory KIRs are known to bind various HLA-A, HLA-B, and HLA-C alleles; however, the ligands for most activating KIRs are unknown. While both KIRs and HLA molecules are highly polymorphic, HLA proteins contain certain shared motifs that mediate KIR recognition. The HLA-C C1 and C2 epitopes are defined by a sequence dimorphism (Lys/Asn) at position 80, situated on the $\alpha 1$ helix near the C-terminal end of the peptide binding cleft, which is complemented by a corresponding dimorphism (Met/Lys) at position 44 of KIR2D isoforms (31, 32). The HLA-A/B Bw4 motif comprises residues 77–83 on the $\alpha 1$ helix of HLA-A and HLA-B molecules, and NK cell specificity is largely determined by identity of the residue at position 80 (33,34). Two domain KIRs recognize the C1 and C2 epitopes whereas three domain KIRs recognize the Bw4 motif.

As with MHC class I proteins, KIR molecules and KIR/HLA combinations are highly correlated with disease susceptibility and outcome (35). While the majority of studies have focused on KIR in HIV-1, a role for KIR in immune responses to many other viruses has

also been established, including hepatitis C virus (HCV) (36), hepatitis B virus (HBV) (37), human cytomegalovirus (HCMV) (38), herpes simplex virus type-1 (HSV-1) (39), and Epstein-Barr virus (EBV) (15). Studies on the role of KIRs in acquired immunodeficiency syndrome (AIDS) have identified the activating receptor KIR3DS1, its paired inhibitory allele KIR3DL1, and HLA-B Bw480I (HLA-B alleles expressing the Bw4 epitope specifically with an isoleucine at position 80) as providing protective effects against HIV-1 pathogenesis. KIR3DL1 (97% identical to KIR3DS1) specifically binds HLA-B Bw480I complexes (40, 41). Due to the close homology of KIR3DS1 to KIR3DL1, KIR3DS1 has been predicted to also recognize HLA-B Bw480I ligands. Evidence for the interaction of KIR3DS1 with HLA-B Bw480I comes from numerous genetic association studies that show that expression of KIR3DS1, either alone or in combination with HLA-Bw480I, is associated with a beneficial outcome during HIV infection. (42–48). These observations are supported by the finding that NK cells expressing KIR3DS1 are preferentially activated and lyse HIV-1-infected target cells in an HLA-B Bw4-80I-dependent manner (44,49). However, demonstrating a direct interaction between activating KIR receptors and HLA/peptide complexes biochemically has remained elusive, at least partly due to the extreme difficulty in expressing soluble forms of activating KIR receptors suitable for biochemical studies, an observation that we can personally attest to.

During HIV-1 infection, mutations that map to T-cell epitopes can also affect recognition of HLA by KIR3DL1. In one study (50), distinct HIV-1 epitopes differentially modulated the binding of KIR3DL1 to HLA-Bw4. Other studies have reported that HIV mutations emerging early during infection within an HLA Bw4-restricted T-cell epitope abrogate binding to KIR3DL1 (51) and KIR2DL1 (52). Furthermore, changing the peptide presented by the HLA molecule can more efficiently abolish the inhibitory response than downregulation of HLA alone (53). These results suggest that detection of T-cell escape variants by NK cells could contribute to the protective effect of the KIR3DL1/HLA-Bw4 compound genotype (50).

Major questions recently resolved or currently unresolved regarding KIR function, potentially addressable by structural approaches, include understanding whether and how differences in peptide sequences presented by MHC class I proteins are discerned, understanding whether and how recognition mechanisms of two- and three-domain KIRs differ, and identifying the ligands for activating KIRs (and why such highly-homologous receptors would have distinct specificities).

The structures of KIR2DL1 in complex with HLA-Cw4 and KIR2DL2 in complex with HLA-Cw3

While the two available KIR2D/HLA complex structures have been around long enough to have been extensively reviewed (54–59), several salient details are worth summarizing to place more recent results in context. The two available KIR2/HLA complex crystal structures, of KIR2DL1/HLA-Cw4 (60) and KIR2DL2/HLA-Cw3 (61), display essentially the same overall arrangement of domains (Fig. 2). The two complexes are arranged with the KIR moieties sitting on HLA in an orthogonal docking orientation similar to that observed in TCR/HLA complexes, though more skewed to the C-terminal end of the groove, with the D1/D2 domains contacting the $\alpha 1/\alpha 2$ helices of the MHC peptide-binding cleft and positions seven (P7) and eight (P8) of the bound peptide (nonamers in both cases). The KIR/HLA interface is dominated by charge complementarity; six loops on the electronegative binding surface of KIR (three from D0, two from D2, and one from the hinge loop connecting these two domains) interact with the electropositive binding surface on HLA, resulting in the formation of a network of hydrogen bonds and salt bridges. Specificities of KIR2DL1 for the C2 epitope and KIR2DL2 for the C1 epitope are achieved through different mechanisms: KIR2DL1 contains a shape-complementary pocket for Lys80 in

HLA-Cw4, with the charged primary amine of the Lys80 side-chain contacting the polar side-chains of Ser184 and Glu187, and the aliphatic portion of the Lys80 side-chain contacting the apolar side-chain of Met44, in KIR2DL1. KIR2DL2, on the other hand, recognizes Asn80, which extends from the surface of HLA-Cw3 to a much smaller degree than Lys80 in HLA-Cw4, through a hydrogen bond to Lys44 of KIR2DL2. In both complex structures, contacts between KIR moieties and the antigenic peptide specific for side-chains are sparse, with tenuous van der Waals contacts made by KIR residue Leu104 in both complexes, and Gln71 in the KIR2DL2 complex, and only to the penultimate residue in the peptide. Mutational analyses have identified the interactions that contribute most to KIR/HLA binding; all of the mutations made to either the receptor or the peptide at positions shown to participate in contacts across the interface greatly reduced or completely abolished binding affinity (62). This low tolerance to mutation at the interface, however, is likely partly a reflection of the low observed equilibrium dissociation constants, in the 10 μ M range, which provide little headroom for quantifying affinity reductions.

Are three domains better than two: KIR3DL1 recognition of HLA-B*5701

The first crystal structure of a three domain KIR, KIR3DL1, was recently determined in complex with HLA-B*5701 (63) (Figs 2 and 3). KIR3DL1 binds to HLA-B*5701 with its D1 and D2 domains positioned over the C-terminal end of the peptide binding cleft in an overall arrangement very similar to that in the KIR2D/HLA complexes. The D0, D1, and D2 domains together trace a zigzag path through KIR3DL1 bound in the complex, which allows D0 to extend down over the edge of the HLA α 1 domain to interact directly with both the α 1 and β 2m domains of HLA-B*5701. The long axis of the D0 domain is oriented almost perpendicular to the long axis of the peptide-binding cleft; this allows KIR3DL1 to make contacts with a relatively conserved region of the peptide-binding platform domain (outside of the polymorphic peptide- and TCR-binding surfaces), and extend to make contacts with invariant β 2m, providing, overall, ~30% of the binding surface area. Since this interaction involves residues conserved on both sides of the interface, a portion of HLA relatively invariant across multiple alleles and a conserved segment of KIR, including many contacts to main-chain atoms, this interaction may be conserved across other three domain KIRs binding to HLA proteins. The surface on the peptide/HLA complex contacting the KIR3DL1 D1-D2 domains is relatively flat and allows for close positioning on HLA-B*5701; in particular, the D2 binding site is highly shape complementary to its cognate surface on HLA, excluding interfacial water molecules, where the D1 binding site is considerably less so. Analogous to the two domain KIR structures, the D1 domain makes contacts with the α 1 helix and the peptide, and the D2 domain makes contacts with the α 2 helix. Key contacts are made to a region of the HLA α 2 helix also relatively conserved across alleles and alanine substitution at any of these KIR D2 domain residues (Tyr200, Phe276, Glu282) or HLA-B*5701 residues (Ile142, Lys146, Ala149) abrogates binding of KIR2DL1 to HLA-Bw480I. Perhaps somewhat surprisingly at first glance, alanine substitutions in D1 (Lys136, Gly138, Ser140, Met165, Leu166, Ala167) at residues that make contact to the Bw4 epitope-defining residues, the epitope that imparts specificity of KIR3DL1 recognition of HLA alleles, did not greatly affect binding. This observation raises questions about the nature of the structural mechanism for specificity of this epitope. In HLA-B*5701, the side-chain of Ile80, a key residue associated with KIR3DL1 recognition, is positioned within a shallow depression ringed by the side-chains of Glu76 and Arg83 from the Bw4 motif on the α 1 helix; Tyr84, also on the α 1 helix; the C-terminal carboxylate and the side-chain of the penultimate residue (SerP8) of the bound peptide (LSSPVTKSF); and the side-chain of Lys146 on the α 2 helix. The side-chain of Ile80 makes only one direct contact to KIR3DL1, a van der Waals bond to the side-chain of Leu166. Otherwise, Ile80 sits underneath a water-filled cavity at the KIR/HLA interface, where the ordered water molecules are also multiply coordinated by the side-chains of Glu76 and Arg83, which penetrate into the water cluster.

Alanine substitutions at any of the residues from the B*5701 α 1 helix ring this water filled cavity (Glu76, Arg79 and Arg83), all three of which also make direct contacts to KIR3DL1, severely reduced or abrogated binding. Alanine substitution at Ile80 also abrogated binding, and mutation to Ile80 to Thr, a common dimorphism found in the Bw4 motif, reduced binding by approximately 40%. The side-chain-specific, direct protein-protein contacts between KIR and HLA involving the Bw4 residues Arg79 and Arg83 are provided by main-chain atoms of KIR residues Gly138 and His278, partly explaining the apparent insensitivity of KIR3DL1 binding to mutations at Bw4 contacting residues.

Observations that KIR3DL1 is capable of considerable discrimination of the identity of the penultimate residue of the MHC-bound peptide (50, 51, 63) are difficult to reconcile with the complex structure. The lone KIR contact with the peptide at position eight (SerP8) is through a single side-chain to side-chain van der Waals contact with Leu166 of the D1 domain; longer P8 side-chains would also potentially be capable of direct interactions back to HLA. Experimental measurements of the effect of P8 substitutions on 3DL1 binding [in either B*5701 (63) or B*5703 (51) backgrounds] show that many replacements were tolerated with relatively small (less than 10-fold) changes on 3DL1 affinity, while others (Gln, Leu, Asp, Lys, Glu) much more greatly reduced affinities. Depending upon rotamer selection, there is room to accommodate various amino acids at P8, since this residue sits at the edge of the KIR/HLA interface with access to solvent. The puzzle is in the apparent lack of a pattern of allowed/disallowed amino acids; charge, hydrophobicity, and side-chain bulkiness do not appear correlated with affinity changes. It is also not immediately apparent how such fine discrimination is possible without a larger number of neighboring contacts to provide the means to discriminate. There is a small pocket immediately adjacent to Leu166, bounded by Ser115, Pro199, and Glu282, that could accommodate longer P8 side-chains, seemingly almost ideal for lysine or arginine substitutions, both of which can be reasonably modeled into the complex; however, lysine is among the most disfavored amino acids. Therefore, the nature of the selection rules and their structural basis remains unclear and merits further structural analyses.

Commentary on the need for experimentally determined structures and the limits of modeling: the role of the D0 domain in KIR3D binding

Prior to the determination of the crystal structure of the complex of KIR3DL1 with an MHC class I protein, an extensive effort was undertaken to model the complex structure (Fig. 4), precipitated by the observation that a set of sequence dimorphisms between KIR3DL1 alleles (*001, *005, and 015) affected binding to a particular MHC class I ligand, HLA-A*2402 complexed with a peptide from the HIV nef2 protein (64). It was clear, prior to these studies, that the D0 domain of KIR3D receptors contributed to MHC class I ligand binding (65). Though details of the interaction were lacking, prior alanine-scanning mutagenesis studies had suggested, for instance, that residues in the 47 to 54 region of the D0 domain affected HLA Bw4 binding (40). [KIR3DS1 does not bind HLA-A24nef likely because the nef peptide has cysteine at P8 which would sterically occlude 3DS1 binding, as discussed in greater detail below.] The effect of the sequence dimorphisms and domain swaps on ligand binding was determined, and antibody binding was used to assess effects on inter- and intra-domain conformational changes. The sum total of these exhaustive mutagenesis and phylogenetic studies generated a list of constraints for positioning models of the D0 domain against the D1 and D2 domains (logically presumed to interact in the HLA complex with KIR3D receptors as they do with KIR2D receptors) and the A24nef ligand. For instance, the KIR D0 47 to 54 region was presumed to directly contact HLA. The D1 and D2 domains of KIR3DL1 (the *015 allele) were modeled on the crystal structure of KIR2DL1 domains D1 and D2 from the HLA-Cw4 complex structure, and the D0 domain was modeled on the crystal structure of the D1 domain of KIR2DL2 from the HLA-Cw3

complex structure, using multiple sequence alignments to identify the best homology model, moderately sophisticated computational modeling tools to adjust loops and accommodate sequence substitutions, and energy minimization to tweak the docking of receptor domains onto the MHC class I ligand. The model was then thoroughly validated by additional mutagenesis/binding studies, producing a compelling structural analysis, one that we felt had gotten it right when we read it. In this model (Fig. 4), the D0 domain packs tightly in the corner between the D1 and D2 domains, with KIR3DL1 perching on the C-terminal end of the peptide-binding groove like a tricorne hat, a quite logical and satisfying solution.

Unfortunately, despite the careful application of logical methodology and exhaustive experimental data, the modeled complex does not recapitulate many of the salient details of the overall domain organization of KIR3DL1, the structure of the D0 domain, or the details of the intermolecular interactions between the D0 domain and the HLA ligand (Fig. 4). For instance, in the complex structure, the KIR D0 47 to 54 region does not contact HLA but points completely away from ligand out into space, forming one of two short α -helices in the D0 domain that were not predicted during modeling. While we hail the efforts of these authors, who did yeoman's duty in this analysis, the pitfalls that likely affected the outcome provide a valuable cautionary tale. First, antibody binding, at best, is a very coarse measure of protein conformation. Second, it is very difficult in forward mutagenesis studies to distinguish between direct effects on contact residues from indirect effects on folding or effects communicated at a distance to contact residues. Also, because of the uneven distribution of binding energy at protein-protein binding sites, direct contact residues may not show dramatic reductions in affinity when mutated to alanines. Third, homology modeling at low sequence identities (the D0 domain was 38% identical to the starting structure used for modeling) is always perilous unless using the most sophisticated computational algorithms available today (and sometimes even then), which are often restricted to dedicated computational biology groups because of the processor resources and arcane computer code required. Protein-protein docking still remains challenging for computational biology, even at the current state of the art, with incomplete tools for validating predicted results, demonstrating the continuing value in determining complex structures experimentally.

Modeling KIR3DS1 interactions to define the unknowns

The physiological ligand for the activating counterpart of KIR3DL1, KIR3DS1, is not yet known but is hypothesized to be HLA-B Bw4 (44, 49), though studies have failed to demonstrate a direct interaction (66, 67). As with most KIR activating/inhibitory pairs, the sequence identity between isoforms is quite high; 3DS1*010 and 3DL1*001 differ at only seven positions: I47V, V92M, S58G, G138W, L166R, P163S, P199L. We have modeled a hypothetical complex between KIR3DS1*010 and HLA-B*5701 by replacing residues corresponding to the 3DS1/3DL1 substitutions in the KIR3DL1*001/HLA-B*5701 crystal structure (Fig. 3). Three of the seven 3DS1/3DL1 substitutions (S58G, V92M, I47V), while predicted to be on the surface of KIR3DS1*010 D0 domain, are distant from the KIR3DL1*001/HLA-B*5701 interface and should not affect MHC class I binding directly or indirectly. Three substitutions (G138W, P199L, L166R) occur at positions where residues make direct contacts with HLA-B*5701 in the KIR3DL1*001 complex structure, and one substitution (P163S) occurs in the D1 loop (161-166). Proline, instead of serine, at this position in 3DL1*001 may serve to reorder the D1 loop and affect the positioning of residue 166, a potential indirect effect on binding. Experimentally swapping the 3DS1*010 residues into 3DL1*001 at the positions directly contacting HLA-B*5701 showed that two substitutions (G138W, P199L) did not result in a substantial decrease in HLA-B*5701 tetramer binding (63). In another study, substitutions in KIR3DL1*015 to those present KIR3DS1*010 either completely abolished (P163S, L166R, P199L) or severely decreased

binding (G138W) to HLA-A24 (64). In the modeled complex, there is space at the interface to accommodate these substitutions without affecting MHC class I interactions. However, replacement of leucine 166 (in KIR3DL1) with arginine (in KIR3DS1) abrogates tetramer binding (63). The side-chain of L166 is positioned directly above the P8 residue; in the KIR3DL1/HLA-B*5701 complex structure, the L166 side-chain makes a van der Waals contact with the hydroxyl of the P8 serine residue. In our model, R166 makes substantial steric clashes with any non-glycine residue modeling into the P8 position, with the caveat that the global KIR/MHC class I domain arrangement is held rigid in these analyses (smallish domain-by-domain rearrangements at the interface might be able to accommodate some of the less severe clashes). A glycine at P8 also sterically clashes, but to a minimal degree that could be accommodated by minimal adjustment of the KIR/MHC class I interaction at the local level. We also note that an alignment of all known KIR3DS1 and KIR3DL1 alleles shows that no residue is wholly unique to either KIR3DS1 or KIR3DL1 (for instance, even R166 can be found in KIR3DL1*054). This observation suggests that not all KIR3DL1 will bind HLA-A/B and that a combination of substitutions likely accounts for the absence of KIR3DS1 binding to HLA-A/B.

HLA-B*5703 structures complexed with three of five known HIV p24-derived T-cell, B*57-restricted epitope peptides are available (68): ISPRTLNAW (ISP), KAFSPEVIPMF (KAF-11), and KAFSPEVI (KAF-8). These peptides vary widely in length and primary sequence and adopt markedly different conformations and associated ordered water structures in the HLA-B*5703 peptide-binding groove. Docking these complex structures onto HLA-B*5701 in the KIR3DL1 complex structure or the KIR3DS1 complex model (a valid exercise, since there are no sequence differences between these two class I sub-alleles within 12 Å of the peptide C-terminal residue directly underlying KIR) shows that the only irreconcilable steric clash with 3DL1 is with the penultimate methionine residue in the KAF-11 peptide, despite the wide range of conformations displayed; all three peptides would be predicted to clash with 3DS1.

The leukocyte immunoglobulin-like receptor (LILR) family

The LILRs [also leukocyte inhibitory receptors (LIRs), immunoglobulin-like transcripts (ILTs), or CD85a-m] are Type I transmembrane glycoproteins with either two or four tandem Ig extracellular domains. Like KIRs, LILRs are encoded within the LRC, consist of both activating and inhibitory forms, and recognize MHC class I proteins (69-72). Inhibitory LILRs signal through ITIMs in their cytoplasmic tails while activating LILRs associate with the ITAM-containing adapter signaling protein FcεRIγ through a positively charged arginine in their TMs (73). These receptors are expressed on NK cells, T cells, granulocytes, plasmacytoid DCs, and cells of the myeloid lineage (74). Of the 13 LILR genes (two of which are pseudogenes), LILRA1, LILRA2, LILRA3, LILRB1, and LILRB2 are known or predicted to bind classical MHC class I proteins, whereas LILRA4, LILRA5, LILRA6, LILRB3, LILRB4, and LILRB5 do not or are not predicted to recognize MHC I (74). LILRB1 and LILRB2 also bind the less polymorphic, nonclassical MHC class I proteins HLA-E, HLA-F, and HLA-G (75-77). HLA-E normally presents a restricted peptide repertoire derived from the leader sequences of other MHC class I proteins (78). HLA-E serves as an indirect check for normal class I expression, but, under conditions of cellular stress, can present peptides derived from heat-shock proteins, pathogen-associated proteins, or the defective processing of antigens (79). HLA-F, while associated with many disease states, is much less understood but has been shown to be a cell-surface marker of activated lymphocytes (80) and to bind free HLA class I heavy chains when is itself in a peptide-free state (81) (no HLA-F-binding peptides have been definitively identified). HLA-G is highly expressed on the trophoblast and plays a key role in maintaining tolerance at the fet-

maternal interface (82), but it has since been demonstrated to also have a tolerogenic function in a variety of pathological conditions (83).

The potential importance of LILRs in immune responses to viral infections beyond their role as class I sensors was suggested by the finding that LILRB1 can act as a receptor for the HCMV protein UL18, a viral HLA class I homolog (69, 84, 85); LILRB1 binds to UL18 with 1000-fold greater affinity than to MHC class I proteins (86). While it might be expected that engaging an inhibitory LILR through a HLA class I homologous viral decoy ligand on the surface of an infected cell would inhibit cell lysis, the role in NK cell mediated lysis of HCMV infected cells is unclear: UL18 has been found to either decrease or increase target cell lysis by NK cells depending on the context (87). However, an evolutionary analysis of primate lineages showed that some NKG2-type NK receptors (discussed below) are evolving under positive selection. Such signatures of positive selection suggest that the protein involved is in genetic conflict with, for instance, a pathogen-associated evasion protein. Following on this observation, a search for a possible viral NKG2 interactor led to the discovery that UL18 also binds to the activating NK receptor NKG2C (88), which complicates the picture of how UL18 expression may affect NK cell activation.

LILRs may also play roles specifically in HIV-1 infections. Although LILRs have been shown to bind many HLA class I alleles, there is evidence that the affinity is influenced by allelic polymorphisms. For example, allelic subtypes of HLA-B*35 can be grouped into Px and Py subsets, differing by as few as a single amino acid, but which differentially influence HIV-1 disease progression: the Px subtype is a strong predictor of accelerated HIV-1 disease progression, while the Py allele has no effect (89). Since the Px and Py subsets are highly homologous, often with completely conserved peptide repertoire specificities, it seems unlikely that differences in disease progression would be attributable to CD8⁺ T-cell responses (90). Supporting the alternative hypothesis that the effect is NK mediated, LILRB2 has been shown to bind more strongly to HLA-B*35 Px than HLA-B*35 Py subtypes as measured by tetramer staining and surface plasmon resonance (SPR) biosensor analyses (91). In this latter study, binding through LILRB2 expressed on DCs to HLA-B*35 Px-bearing cells resulted in decreased maturation and cytokine secretion. In a similar vein, HLA-B*57 and HLA-B*27, two alleles that have consistently been associated with better control of HIV-1 disease progression (92), have been shown to have the weakest binding to LILRB2 when compared to over 90 other HLA class I alleles (93). As seen with KIR recognition of HLA, peptide variation may also modulate LILR recognition of MHC class I proteins. In one example, a typical escape mutation in an HLA-B*2705-restricted cytotoxic T-cell epitope from HIV-1 gag (KRWILGLNK, escape: L6M) was shown to substantially enhance LILRB2 binding of HLA-B*2705 tetramers presenting the mutant peptide (94). While these studies are highly suggestive, they have been performed either with DCs or biochemically, so direct associations between AIDS disease progression and LILRB2 signaling on NK cells remains to be demonstrated.

Structures of the LILRB1/HLA-A2 and LILRB2/HLA-G complexes: a conservative viewpoint

The broad specificity of LILRB1 and LILRB2 for numerous HLA alleles is explained by the conserved nature of their recognition sites on MHC class I proteins (Fig. 2). While KIR receptor footprints encompass the highly polymorphic $\alpha 1/\alpha 2$ platform domain of MHC class I proteins, LILRB1 and LILRB2 bind to a surface composed of sections of the relatively non-polymorphic $\alpha 3$ domain and the invariant β_2m light chain (74). The complex crystal structures for the D1-D2 domains of LILRB1 and LILRB2 bound to HLA-A2 and HLA-G, respectively, show very similar binding modes, with two main interaction surfaces: D1 makes contacts with the HLA $\alpha 3$ domain, while the hinge region linking the two domains makes contacts with β_2m (95, 96). LILRB1 and LILRB2 interactions with the MHC class I $\alpha 3$ and β_2m domains bury roughly comparable surface areas at the interfaces,

with more area buried at the β_2m interfaces in both examples but with binding energy more evenly distributed between the α_3 and β_2m interactions [LILRB2: 471 Å buried on α_3 ($\Delta G_{\text{calc}} = -3.2$ kcal/mol), 635 Å² on β_2m ($\Delta G_{\text{calc}} = -4.1$ kcal/mol); *versus* LILRB1: 272 Å² buried on α_3 ($\Delta G_{\text{calc}} = -1.9$ kcal/mol), 584 Å² on β_2m ($\Delta G_{\text{calc}} = -1.8$ kcal/mol); values calculated with PISA (97)]. The interesting observation that LILRB2 but not LILRB1 can bind to β_2m -free HLA heavy chains (14, 19) is not explained by the relative surface areas buried, but it is potentially explained by the difference in calculated solvation free energies of binding (ΔG_{calc}), a function of the nature of the interactions within the interfaces and not just the total area buried. LILRB1 and LILRB2 are oriented head-to-tail with HLA in the complexes, with their N-terminal domains pointed towards the C-terminal, membrane proximal domains of the HLA protein. This orientation is consistent with a *trans* mode of binding, where the receptor on the effector cell engages an HLA molecule on the target cell. While the LILR D1 domains do extend below the plane of the MHC α_3 domains in the complexes, the eight-residue spacer sequence between the MHC α_3 and TM domains is long enough to allow LILRs to fully clear the membrane without canting over the MHC protein in this binding mode. Complexation with HLA triggers changes within the interdomain angle of LILRB1 (shifts of 14 to 19°) which are not observed in LILRB2 during binding (85, 95, 96, 98, 99). In general, however, more extensive conformational changes are seen in LILRB2 during complex formation than in LILRB1. For example, free LILRB2 contains one 3_{10} helix (residues 52 to 55) involving residues at the binding interface; upon complex formation, the interface is restructured with two 3_{10} helices (residues 46 to 50 and 53 to 57). Overall, the structure of LILRB2 in the complex state most closely resembles LILRB1 in the free state. However, these conformational changes presumed to occur concurrent with binding may also reflect the very different crystallization conditions used for the various proteins and/or the process of crystallization selecting particular conformers out of a larger ensemble of conformers accessible to flexible proteins.

While the LILR story appears to have reached consensus, with recognition apparently focused on conserved elements of MHC class I proteins, there is evidence that HLA polymorphisms and peptide identity do play a role in modulating LILR binding (94, 100). Quantitative SPR binding analyses have confirmed that the membrane-distal D1 and D2 domains are primarily responsible for ligand binding (86, 95). However, the lesson of the KIR2D versus KIR3D interactions should not go unheeded; until structures of intact four-Ig domain LILRs, alone and in complex with HLA ligands, are available, the role that the membrane-proximal D3 and D4 domains play in recognition remains formally unknown, with the potential that these domains play minor modulatory or selective roles in binding, possibly in surprising ways.

LILRB1/UL18: recognizing a mimic is easy

HCMV UL18 is a Type I transmembrane glycoprotein homolog of human MHC class I proteins that analogously associates with β_2m and binds peptides within a groove formed by UL18's version of an α_1/α_2 platform domain, with the major difference that UL18 is much more heavily glycosylated than MHC class I proteins (101–103). Despite sharing only ~21% sequence identity with MHC class I molecules, the crystal structure of a minimally glycosylated UL18 construct bound to an actin-derived peptide (ALPHAILRL) in complex with the D1-D2 ectodomain of LILRB1 (84) shows a high degree of structural homology to the LILR/classical and LILR/nonclassical MHC class I complexes at the levels of overall domain organization, contact surfaces, tertiary and secondary structure. However, the orientation between LILR D1-D2 domains with respect to each other differs in detail, with LILRB1 adopting an interdomain angle in the UL18 complex intermediate between that in HLA-A2 complex and LILRB2 in the HLA-G complex. As in the LILRB1/HLA-A2 complex, the linking hinge between the D1 and D2 domains contacts β_2m [497/519 Å²

buried on β_2m in the two complexes in the crystallographic asymmetric unit; values calculated with PISA (97)], while the D1 domain contacts the MHC α_3 domain (579/595 Å² buried on α_3). The nature of the intermolecular contacts between β_2m and the LILRB1 D1-D2 linking hinge were very similar in both the LILRB1/UL18 and /HLA-A2 complexes, but the heavy chain/D1 interactions differed, effectively creating a more favorable binding interface for the latter, including additional salt bridges (two to HLA-A2 *versus* nine/eight to UL18), hydrogen bonds (three to HLA-A2 *versus* nine/seven to UL18), and van der Waals contacts, contrasting the van der Waals-dominated HLA-A2/D1 interface. Overall, recognition of UL18 by LILRB1 is driven by interactions involving the UL18 heavy chain, while detection of HLA is driven by recognition of the invariant β_2m light chain. Of course, this results because HCMV can only access the UL18 sequence, not the host-encoded β_2m sequence, to evolve a tighter-binding LILR decoy ligand. Known variable regions within the UL18 α_1 domain previously predicted to interact with LILRB1 were not observed to do so in the complex crystal structure (104,105). It may be possible that residues in the LILRB1 D3 or D4 domains could provide such contacts, not observed in the crystal structure because these domains were not included in the crystallization construct. However, analytical ultracentrifugation studies (86) have suggested that LILRB1 adopts an extended structure that would require an unlikely bend to reach back and make additional UL18 contacts (the C-terminus of the LILR D2 domains in the complexes all point completely away from the ligand proteins). Furthermore, the affinities for UL18 of LILRB1 D1-D2 constructs *versus* intact ectodomain D1-D4 constructs did not differ significantly (86), all arguing that the D1-D2 complex structure revealed all the details of the interaction.

The extracellular domain of UL18 has 13 predicted N-linked glycosylation sites (106) and, when expressed in mammalian cells, is heavily glycosylated (~55% carbohydrate by weight). A fully glycosylated UL18 was modeled bound to LILRB1 (84). In this model, the UL18 heavy chain was completely shielded by carbohydrate except for two regions; the binding site for LILRs and the docking site for β_2m . This observation led to the hypothesis that UL18 evolved a glycan shield to prevent binding of other mediators of immunity, such as MHC class I binding CD8⁺ T cells or antibodies, while preserving the binding surface for LILRs. This strategy parallels that employed by other viruses, notably the comparably-glycosylated HIV envelope protein.

NKG2-CD94 heterodimers

Members of the NKG2 family of C-type lectin-like receptors, expressed on NK cells and subsets of CD8⁺ T cells, include NKG2A, NKG2B, NKG2C, NKG2E, and NKG2H, all of which obligatorily dimerize with CD94 (which also has a C-type lectin-like fold) (107–110). NKG2-CD94 heterodimers recognize HLA-E molecules (111–114) and are encoded by genes found within the NK complex on chromosome 12p13 (115). NKG2A/B are inhibitory receptors that contain ITIMs in their cytoplasmic tails (116); NKG2C/E/H are activating receptors that associate with DAP12 through a positively charged residue in their transmembrane region (117). The nonclassical MHC class I protein HLA-E is distinct from the classical class I proteins, in that it binds a restricted repertoire of peptides excised from the leader peptides of classical and nonclassical (HLA-G) MHC class I proteins (118). Because HLA-E expression is dependent on the expression of other HLA class I molecules (classical MHC class I and HLA-E proteins do not fully fold and express on the cell surface without binding an antigenic peptide), recognition of HLA-E by NKG2A/C/E-CD94 receptors allow NK cells to monitor the expression of other MHC class I molecules on the cell. The interactions between the various NKG2-CD94 receptors and HLA-E/peptide complexes span a wide range of affinities, with patterns that suggest the system has been tuned to produce particular outputs (109). Responses of NK cells to viral infections in different contexts support this idea. For example, loss of the inhibitory NKG2A signal due

to downregulation of HLA-E by vaccinia results in NK-mediated lysis of virus-infected cells (119). In contrast, infection with hantavirus (120) and HCMV (121) results in upregulation of HLA-E on infected cells, which may be expected to thwart NK cell surveillance against virus infection due to signaling through the inhibitory NKG2A receptor. However, during hantavirus and HCMV infection, there is a specific expansion and persistence of activating NKG2C⁺ NK cells (122) and, with hantavirus, a decrease in the numbers of inhibitory NKG2A⁺ NK cells (120).

The only NKG2 ectodomain structures currently available including the complete recognition module (NKG2 moiety + CD94) are two, essentially identical, contemporaneous structures of NKG2A-CD94 bound to HLA-E (88,123) (Fig. 2) and an earlier structure of NKG2A-CD94 alone (107). These structures are important for revealing views of NKG2 C-type lectin-like folds, 'lectin-like' in the sense that, while the fold is retained, specific structural elements necessary for lectin activity have been lost, allowing these receptors to target protein ligands as opposed to carbohydrates. In the complexes, CD94 binds over the α 1 helix of HLA-E and contributes all contacts to the C-terminal portion of the peptide, while NKG2A sits over the over the α 2 helix, an orientation very reminiscent of TCR/MHC interactions. Recognition of the C-terminal half of the peptide is important, since almost all of the limited variation among HLA-E-restricted peptides is localized to the C-terminal residues, showing that CD94, the invariant element in NKG2 heterodimers, directly reads out peptide sequence differences, which can result in moderate affinity differences (109). Modulation of affinity differences between NKG2-CD94 family members is achieved primarily through adjustments to the orientation of the two halves of the heterodimer; residues at the heterodimer interface also display the highest signatures of evolutionary positive selection, consistent with the idea that positive selection due to genetic conflict with a pathogen-associated evasion molecule would drive escape from the interaction.

In addition to these ectodomain structures, there is a rather unique structure available for the heterotrimeric assembly of TM domains from DAP12 and NKG2C, determined in its membrane-embedded form by NMR spectroscopy (120,167). In this structure, the intramembrane contacts between TMs are dominated by a network of electrostatic interactions between five polar residues. Since such polar/electrostatic TM interactions are often found between activating immunoreceptors and their cognate adapter molecules, this structural motif potentially serves as an example of a key molecular organizing element.

Structural and biochemical studies have raised, rather than addressed, two puzzling aspects of the NKG2-CD94 family, pertaining to the NKG2E and NKG2F isoforms. NKG2E-CD94 has been expressed recombinantly, purified, and used in quantitative SPR binding analyses, displaying a pattern of affinities seemingly tuned, through unique structural mechanisms, to match those of the inhibitory receptor NKG2A-CD94 (109). However, despite the presence of a positively charged residue (lysine) in its TM, NKG2E-CD94 has not been demonstrated to interact with DAP12 (a requirement for activating NKG2 cell-surface expression) or to be found on the surface of any cell type, under any condition (59). NKG2F, on the other hand, which contains a cytoplasmic ITIM-like sequence, is missing a large, otherwise-conserved section in the C-type lectin-like domain, which, from a structural perspective, seems indispensable for folding (124). NKG2F has also not been found on the surface of any cell type, under any condition. Therefore, the question arises as to whether these two isoforms are expressed under any conditions and, if so, how the structural hurdles to their expression are overcome. If they are not functionally expressed, the question arises as to why they are retained in the genome in a form that is potentially functional, at least for NKG2E.

Receptors specific for non-MHC class I ligands

NKG2D homodimers

NKG2D is a Type II transmembrane C-type lectin-like protein encoded by the KLRK1 (killer cell lectin-like receptor subfamily member 1) gene on chromosome 12p12-13 and is broadly expressed on NK cells and cytotoxic T cells (116, 125). In humans, NKG2D transmits activating signals through the adapter molecule DAP10 upon recognition of stress induced MHC class I chain-related molecules (MICA/B) and UL16-binding proteins (ULBP1-6) (126). NKG2D ligands are highly polymorphic and resemble MHC class I molecules in terms of domain organization and fold. Unlike MHC class I proteins, NKG2D ligands do not associate with β_2m or antigenic peptides (127–130). Despite its name, NKG2D is quite distinct from the rest of the NKG2 family, showing limited sequence identity to the otherwise closely related family members, forming homodimers rather than obligate heterodimers with CD94, and using DAP10 as an adapter rather than DAP12. NKG2D structural analyses are perhaps most valuable for revealing a novel mechanism enabling functional recognition polyspecificity, ‘rigid adaptation’ (23, 131, 132), and are otherwise quite near-and-dear to our hearts but have been previously reviewed and analyzed almost *ad nauseam*, so we do not discuss it further here.

Natural cytotoxicity receptors (NCRs): the direct approach

NCRs were discovered as the result of a hunt expressly dedicated to finding HLA-independent activating receptors (133). The three requirements that putative NCRs needed to meet were (i) expression should be restricted to NK cells, (ii) monoclonal antibody (mAb)-mediated crosslinking targeting the candidate NCR should trigger NK cell lysis in a redirected killing assay, and (iii) mAb-mediated masking of the receptor should inhibit NK cell mediated cytotoxicity. Currently, this group consists of the activating receptors NKp30, NKp44, and NKp46. NKp46 maps to chromosome 19q13.42 in the LRC, whereas NKp44 and NKp30 are found on chromosome 6p21.3. NCRs are Type 1 transmembrane glycoproteins, members of the Ig superfamily, and, though they do not share close sequence homology with one another or with other NK receptors, they are structurally quite similar to KIRs and LILRs. NKp46 and NKp30 are expressed on resting and activating NK cells, and NKp44 is selectively expressed on IL-2-activated NK cells (134,135). NCRs contain a positively charged residue in the TM that enables them to bind to adapter signaling molecules: DAP12, CD3 ζ , and Fc ϵ RI γ (136–137).

NCRs have received much attention for their involvement in tumor cell lysis; however, the known and characterized ligands for these receptors are often viral in origin and include the IV, Newcastle disease virus (NDV), and Sendai virus (SV) hemagglutinins (HAs) (14, 138, 139), and HCMV pp65 (140). NCRs have also been reported to mediate protective responses during infections with HCV (141,142), HSV (143), Ebola and Marburg viruses (144), Dengue and West Nile viruses (145), and vaccinia virus (146), though the ligand/s targeted in these contexts remain unknown and even controversial. Crystal structures for the extracellular domains of NKp46 (147, 148), NKp44 (149), and NKp30 (150) have been determined. However, no ligand-bound complex structures of NCRs have been reported. Therefore, the polyspecificity mechanisms through which these receptors are able to bind multiple, distinct ligands, including those upregulated during tumorigenesis or infection, or encoded by pathogens, is not known.

The ectodomain of NKp30 consists of a single I-type Ig domain. The I-type domain is structurally intermediate between the V and C-type domain architectures originally identified in antibody structures. Despite limited sequence similarity, NKp30 is structurally homologous to the cell adhesion molecule Coxsackievirus and adenovirus receptor (CAR)

(RMSD = 2.2 Å) and the T-cell coreceptors cytotoxic T-lymphocyte antigen-4 (CTLA-4) (RMSD = 2.6 Å) and CD28 (RMSD = 2.6 Å). NKp30 has been postulated to bind a wide array of ligands including HCMV pp65 (140), Duffy-binding-like domain 1α of *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) (151), leukocyte antigen-B-associated transcript 3 (BAT3) (152), heparan sulfate and heparin (153), and the B7 family member B7H6 (154), the latter biochemically verified through detailed epitope mapping and mutational analyses (150). The B7H6 binding site on NKp30 is proposed to occupy the structurally analogous position on CD28 family members that serves as the common, promiscuous binding site for B7 ligands (155,156). NKp30 is also the target of an immunoevasion mechanism mediated by the HCMV tegument protein pp65. pp65 binds to NKp30, causing it to dissociate from its signaling adapter, CD3ζ, thus preventing the transduction of activating signals (140).

NKp44 consists of a single V-type Ig domain in the extracellular region. A unique feature in the otherwise typical two β-sheet sandwich V domain structure is the presence of a second disulfide bond, which stabilizes the 33-51 β-hairpin. The abutment of the β-hairpin with the 87-112 β-hairpin, a region corresponding to the complementary determining region of antibody and TCR variable domains, forms a prominent groove on the protein surface, proposed as a ligand-binding site. One side of the groove is lined with the side-chains of four basic residues, which creates a positive face, while the other side is neutrally charged.

NKp46 consists of two C2-type Ig extracellular domains, D1 and D2 (148). The two closest homologs to NKp46 are KIRs, which share, at most, ~37% sequence identity, and LILRs, which share ~45% sequence identity, when comparisons are made domain-by-domain. Structural comparisons of NKp46 with KIR2DLs, LILRB1, and LIRB2 show considerable structural homology: superposition RMSDs of 1.8 Å to 2.3 Å superimposed as intact D1-D2 units and RMSDs of 0.9 Å to 1.4 Å when D1 or D2 domains are superimposed individually. The interdomain hinge angle (~85°) and D1/D2 domain orientation are also conserved between NKp46 and KIRs/LILRs. However, comparisons between the binding sites of KIR or LILR and NKp36 show that the residues involved in ligand recognition are not conserved, consistent with the observation that NCRs do not bind MHC class I proteins.

NKp44 and NKp46 both bind IV, NDV, and SV HAs through interactions largely mediated by terminal sialic acid groups (the cellular receptor for HAs) on N-linked NCR carbohydrates. Recognition of HA by NKp46 relies heavily on the O-linked glycan on the stalk region between the Ig ectodomains and TM, particularly at residue Thr225. In NKp46, Thr225 is also responsible for recognition of undefined tumor ligands, suggested to act by maintaining the correct conformation of the binding site (157). This generalized mechanism of viral HA recognition may allow NKp44 and NKp46 to bind HAs from a wide range of viruses due to the conserved function of HAs to bind sialic acid-containing glycoproteins (158). This recognition mechanism is unlikely the whole story, since binding in these cases does not formally rely on a structured ectodomain, merely the property that these NCRs are glycoproteins with terminal sialic acid groups. IV has also developed an immunoevasion strategy to counter this defense. While HA is an activating ligand of NKp44 and NKp46 when expressed on the surface of infected cells, IV infected cells also release soluble HA that can be taken up by NK cells, presumably through NCR interactions. This results in lysosomal degradation of the CD3ζ chain, preventing the transduction of activating signals from NKp44 and NKp46 (159). This immune evasion mechanism likely contributes to the pathogenesis of IV.

Coda

Fine-tuning of NK receptor signaling across the array of often cross-reactive (recognizing common ligands), often opposing receptors seems to enable a precise discrimination of even

slightly altered classical and nonclassical MHC class I expression levels on cells stressed as a result of any of a number of pathologies. This process is complemented by altering the repertoire of peripheral NK cells, defined by the subsets and patterns of receptors expressed on any given NK cell, to generate maximally effective responses to infection or other pathology. Unfortunately, neither the mechanism of signal integration nor the process of NK cell development and selection are fully understood. For instance, NK cells pass through a process known as ‘licensing’ during development, an ‘MHC-dependent process that results in enhanced capacity of NK cells to be activated when stimulated through an activation receptor’ (160). Though first described some time ago, the exact role and outcome of licensing on NK cell activity remains an active topic of discussion (160,161), illustrating how much there is yet to learn about NK cell development and receptor signaling mechanisms. Structural studies, stepping in where they are most useful, have begun to address the details of the signal transduction process by revealing the minutiae of the TM interaction between activating receptors and adapter proteins, but much ground remains to be covered. Biophysical results, particularly recent ones, have provided crucial insights into the structural relatedness of NK receptors to the larger landscape of known protein structures and snapshot views of the recognition events mediating NK activation and viral immunoevasion. A common theme clearly employed by NK receptors to recognize diverse, polymorphic ligands is to focus attention on the most structurally conserved elements, although variations on this theme clearly play fundamental roles in the process of generating functional polyspecificity. Parsing evolutionary relationships on the basis of structural results is always dicey, but a remarkable observation bears further comment: classical and nonclassical MHC class I proteins are multifarious recognition platforms for a whole host of receptors, with nearly every accessible surface involved in some binding event (Fig. 2, which does not even include maps of the binding sites of TCRs or CD8). While they likely evolved as ligands for TCRs, based on the ancestry of this immunological mechanism, more recent immunorecognition systems, particularly those employed by NK cells (and the viruses attempting to avoid them), have made effective use of MHC class I proteins as ligands. Undoubtedly, this places new constraints on the future evolution of MHC class I proteins, providing ample grist for many more reviews on this subject in the future.

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Fig 1. The structure and domain organization of MHC class I proteins

The structure of HLA-B*5701 is shown, in two orthogonal views (A and B), as a ribbon representation (α -helices shown as coils and β -strands shown as arrows) with a semi-transparent rendering of the molecular surface superimposed. The α -chain is colored blue-to-red from N- to C-terminus and the light chain (β_2m) is colored dark gray. Domains are labeled. The peptide is shown in a CPK representation, with alternating residues colored purple or orange. The view in (B) looks down onto the top of the protein, the view seen from the perspective of an incoming TCR.

Fig 2. Interaction footprints for representative NK receptors on MHC class I proteins

Molecular surface representations of the class I ligand protein are shown, colored light gray for the heavy chain (α -chain) and dark gray for the light chain (β_2m); the bound antigenic peptide is shown as an orange squiggle. In the top row, the NK receptor is shown in ribbon representation, colored purple, with α -helices indicated as coils and β -strands indicated as arrows. The middle and bottom rows show the class I protein without the receptor, in two different orthogonal views. The interaction footprint of the receptor on the class I ligand is colored aqua, with the receptor domains contributing the contacts labeled in the middle and bottom rows. Contacts to the peptide are also colored aqua.

Fig 3. The KIR3DL1/HLA-B*5701 interface

(A) An overview of the complex is shown, with both class I ligand and KIR receptor shown in ribbon representations; a semi-transparent rendering of the molecular surface of KIR3DL1 is superimposed. The peptide is shown in CPK representation, colored by atom type (carbon: gray; oxygen: red; nitrogen: blue). (B) A zoomed-in view of the interface, centered on I80 in HLA-B. Only segments of the protein backbone ribbons and the side-chains of key residues [shown in licorice-stick representation, colored by atom-type as in (A) and labeled] are shown for clarity. The segment of the HLA-B heavy chain corresponding to the Bw4 epitope is colored gray. Direct contacts between Val166 in KIR3DL1 and Ile80 in HLA-B and P8 in the antigenic peptide are indicated with yellow dotted lines. The cluster of ordered water molecules in a pocket between the D1/HLA interface is shown as red spheres. (C) A model of the effect of substituting Val166 with arginine, the key 3DL1/3DS1 sequence difference. The side-chain of Arg166, placed in the least disallowed rotamer, is shown in CPK representation, colored by atom-type as in (A). The molecular surface of HLA-B*5701 is colored light gray for the platform domain, black for the peptide and red for P8 in the peptide. The steric clash is readily apparent, shown by the penetration of the Arg166 side-chain through the molecular surface.

Fig 4. Comparison of the KIR3DL1*001/HLA-B*5701 complex structure with the KIR3DL1*015/HLA-A24 model

(A) A superposition of KIR3DL1*001/HLA-B*5701 and KIR3DL1*015/HLA-A24 is shown as a ribbon representation. KIR3DL1*001 is colored by domain (D0 in light green, D1 in aqua, and D2 in dark green); HLA-B*5701 is colored light grey with bound peptide in orange; KIR3DL1*015 is colored by domain and labels are qualified by asterisks (D0* orange, D1* red, D2* blue); HLA-A24 is colored dark grey with bound peptide in yellow. (B) The footprint of KIR3DL1*001 is shown on the molecular surface of HLA-B*5701 (top figure) and the footprint of KIR3DL1*015 is shown on the molecular surface of HLA-A24 (bottom figure) as viewed looking down on the peptide binding platform. Individual domain contacts are colored as in (A) with the exception the D1-D2 loop of KIR which is colored dark blue. (C) Ribbon representations of KIR3DL1*001 and KIR3DL1*015 are shown as viewed from above [rotated 90° from the position shown in (A)]. Individual domain contacts are colored as in (A).

Table 1

List of human NK cell receptors: structures, signaling, and viral involvement.

activating receptor	PDB	ligand	signaling	virus	references
NKG2D	1HQ8, 1HYR (NKG2D/MICA), 1KCG (NKG2D/ULBP3), 1JSK (NKG2D/Rae-1β)	MICA/B, ULBP1-6	DAPI10	HCMV, KSV, HBV, HIV-1, IV	126, 128, 129, 162–165
NKG2C-CD94	2L35 (DAP12/NKG2C)	HLA-E	DAPI12	HCMV, HBV, HCV, hantavirus	112, 120, 122, 142, 166, 167,
NKp46 (NCR1)	1P6F, 1OLL	HA, heparan sulfate/heparin	FceRIγ, CD3ζ	Influenza, vaccinia, HCV, HSV, NDV, SeV	14, 139–143, 146–148, 153
NKp44 (NCR2)	1HKF	HA, heparan sulfate/heparin, E-protein, PCNA, bacterial cell wall elements	DAPI12	Influenza, vaccinia, HSV, NDV, SeV, Dengue, West Nile, HIV-1	138, 139, 143, 146, 149, 153, 168
NKp30 (NCR3)	3NOI	B7H6, BAT3, HCMV pp65, heparan sulfate/heparin, PLEMP-1	FceRIγ, CD3ζ	Influenza, vaccinia, HCV, HSV, HCMV, Ebola, Marburg	140, 142–144, 146, 150–154, 169, 170
NKp80	---	Activation-induced C-type lectin	AICL	KSV	163, 171
KIR2DS1	---	HLA-Cw2,4,5,6 (C2 epitope)	DAPI12	EBV	15
KIR2DS2	1M4K	---	DAPI12	HCV, HSV-1	172, 173
KIR2DS4	3H8N	HLA-Cw4	DAPI12	HCV	174–176
KIR3DS1	---	HLA-Bw4?	DAPI12	HIV	42, 44, 49
KIR2DL4	3H8N	HLA-G	FceRIγ, 1 ITIM		177
inhibitory receptor	structure	ligand	signaling	virus	references
NKG2A/B-CD94	1B6E (CD94), 3BDW (CD94/NKG2A), 3CDG and 3CII (CD94/NKG2A/HLA-E)	HLA-E	1 ITIM	HCMV, HIV-1, HCV	88, 107, 112, 123, 178, 179, 180
KIR2DL1	1NKR, 1IM9 (KIR2DL1/HLA-Cw4)	HLA-Cw2,4,5,6 (C2 epitope)	2 ITIM	EBV	15, 60, 181
KIR2DL2	2DL1, 2DL2, 1EFX (KIR2DL2/HLA-Cw3)	HLA-Cw1,3,7,8 (C1 epitope)	2 ITIM	HCV, HSV-1	61, 172, 182, 183
KIR2DL3	1B6U	HLA-Cw1,3,7,8 (C1 epitope)	2 ITIM	HCV	184, 36
KIR3DL1	3VH8	HLA-A, B (Bw4 epitope)	2 ITIM	HIV	49, 63, 185
LILRB1 (ILT2)	1UFU, 1VDJ, 1G0X, 1P7Q (LIR1/HLA-A2), 3D2U (LIR1/UL18)	HLA class I, HLA-G, HLA-F, HLA-E, UL18	4 ITIM	HCMV, HIV-1	75, 84, 85, 95
LILRB2 (ILT4)	2GW5, 2DYP (LIR2/HLA-G)	HLA class I, HLA-G, HLA-F, HLA-E, UL18	3 ITIM	HCMV, HIV-1	75, 96, 99