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Variable NK Cell Receptors Exemplified by Human KIR3DL1/S1

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Variegated expression of variable NK cell receptors for polymorphic MHC class I broadens the range of an individual’s NK cell response and the capacity for populations and species to survive disease epidemics and population bottlenecks. On evolutionary time scales, this component of immunity is exceptionally dynamic, unstable, and short-lived, being dependent on coevolution of ligands and receptors subject to varying, competing selection pressures. Consequently these systems of variable NK cell receptors are largely species specific and have recruited different classes of glycoprotein, even within the primate order of mammals. Such disparity helps to explain substantial differences in NK cell biology between humans and animal models, for which the population genetics is largely ignored. KIR3DL1/S1, which recognizes the Bw4 epitope of HLA-A and -B and is the most extensively studied of the variable NK cell receptors, exemplifies how variation in all possible parameters of function is recruited to diversify the human NK cell response.

Species-specific evolution of variable NK cell receptors

Some NK cell receptors for MHC class I evolve rapidly, which became apparent from species comparison (13) (Fig. 1). The rodent NKC encodes variable Ly49 receptors that diversify NK cell function in rats (14) and mice (15), while the LRC family of killer cell Ig-like receptors (KIR) provides a comparable system for humans and other simian primates (16). Mammalian species having only single-copy Ly49 and KIR genes can survive and flourish (17), but no species has yet been found to have both variable Ly49 and KIR (Fig. 1A). In the context of variable KIR, human Ly49 is a single-copy pseudogene (18); in the context of variable Ly49, the mouse KIR locus left the LRC for the X chromosome (19), where it comprises two genes: one expressed by NK cells and T cells (20), the other by brain cells (21). Such contrasting situations point to past crises in mammalian evolution when species-specific expansion of a new type of NK cell receptor accompanied extinction of an older form.

Further evidence for independent evolution of MHC class I receptors is seen within the LRC. Flanking the KIR locus is the gene family encoding the leukocyte Ig-like receptors (LILR) (22). Of these, LILRB1 is an NK cell receptor that binds to the more conserved domains (Ig-like α1 and β2-m) of MHC class I (12), whereas the variable α1 and α2 domains of MHC class I are the target for KIR (11). Embedded within the LILR locus is a KIR pseudogene (23), KIR3DX, which diverged from genes of the functional KIR locus ~135 million years ago, before the radiation of placental mammals (24). Cattle, even toed ungulates, are the only nonprimates known to have variable KIR (20), but in cattle it was KIR3DX that became the variable gene family, whereas KIR3DL, the ancestral founder of the variable primate KIR, remained a single-copy gene (24) (Fig. 1A).

Common to mice (25) and humans (26) are NKC-encoded MHC class I receptors comprising heterodimers of CD94 and an NKG2 family member. These CD94:NKG2 receptors recognize complexes of a conserved nonclassical class I molecule (mouse Qa1 or human HLA-E) and peptides derived from the leader sequences of other MHC class I molecules (10). Like KIR (11), the CD94:NKG2 receptors interact with
the upward face of the $\alpha_1$ and $\alpha_2$ domains and are sensitive to residues of the bound peptide (27, 28). Although CD94, NKG2, and HLA-E are conserved in humans (29), analysis of the gray mouse lemur showed the potential of CD94:NKG2 to be a variable NK cell receptor (30). This prosimian primate has single $\text{Ly}_49$ and $\text{KIR}$ genes, but three CD94 genes and eight (five expressed, three pseudogenes) NKG2 genes.

Equally distinctive are the lemur’s MHC class I genes; while four class I pseudogenes remained part of the MHC, the cluster of six functional class I genes left the MHC for a different chromosome (30).

Coevolution of MHC class I and KIR in simian primates

That prosimians and most nonprimates have just one KIR gene shows that the diverse family of human KIR genes originated during simian primate evolution, following their separation from prosimians $\sim 58–69$ million years ago (31) (Fig. 1B). Simian primates comprise New World monkeys, Old World monkeys, lesser apes (gibbons), and hominids (great apes and humans). Distinctive lineages of human KIR recognize epitopes carried by different HLA class I molecules. Notably, lineage II KIR recognize some HLA-A and B allotypes, and lineage III KIR recognize HLA-C and some HLA-B allotypes (32–34). These functional interactions are the result of the coevolution of ligands with receptors during simian primate diversification (35).

Lacking counterparts to HLA-A, -B, and -C, New World monkeys have distinctive MHC class I and KIR, showing that they took a different evolutionary tack from that followed by other simian primates (36, 37). The abundance of Old World monkey MHC class I genes resembling either HLA-A or HLA-B (38, 39) correlates with increased numbers of lineage II KIR genes (40–42). Associated with the emergence of MHC-C in hominids is a multiplicity of lineage III KIR genes (34). While KIR evolution in Old World monkeys and hominids is marked by gene expansions, the lesser apes took a different road (43). Although having orthologs of most human class I genes and pseudogenes, gibbon MHC haplotypes lack an ortholog of HLA-G that provides the ligand for KIR2DL4 (44). Correlating with this absence, KIR2DL4 has been either deleted from gibbon KIR haplotypes or disabled. Gibbon MHC haplotypes also lack an HLA-C ortholog, and correspondingly gibbon KIR haplotypes lack the multiplicity of lineage III KIR genes characterizing species with MHC-C (43). The only gene spared from the deletions and mutations that diminished and disordered the gibbon KIR locus is that of lineage V encoding KIR3DL3, for which ligands and function are not known (45, 46). As a potential clue, the inhibitory signaling motifs of gibbon KIR3DL3 appear stronger than their human counterparts (43).

Although first studied in the context of tumor immunity (1, 2), 40 y of research have firmly placed NK cells in the response to infection (47), where they cooperate with dendritic cells (48). NK cells also have a seminal role in reproduction through cooperation with extravillous trophoblast to enlarge maternal blood vessels that supply the placenta and nourish the fetus (49). All such cellular interactions of NK cells can be influenced by KIR engagement of MHC class I. Whereas most cell types express HLA-A, -B, and -C, only HLA-C is expressed by extravillous trophoblast (50). It is also the only normal tissue to express HLA-G, which binds avidly to LILRB1 (51) and interacts with lineage I KIR2DL4 in endosomes (44).
The tissue distributions of HLA-C and -G, and the fate of the gibbon KIR locus in their absence (43) suggest that selective pressures from reproduction induced MHC-C to evolve away from its MHC-B–like ancestor: to be expressed on trophoblast and recognized by the lineage III KIR preferentially expressed on uterine NK cells (52). In this model, HLA-C interactions with lineage III KIR are subject to selection pressures from both immunity and reproduction, whereas the interactions of lineage II KIR with HLA-A and HLA-B evolve principally under selection by infection. As a consequence HLA-A and HLA-B evolved to be exceptionally variable, as has lineage II KIR3DL1/S1 that recognizes a broad range of HLA-A and -B allotypes and has variability that does not stand out from the mass of human genes (Fig. 2). Because of these features, the genetic and functional properties of KIR3DL1/S1 have been most extensively studied, making it an exemplary variable NK cell receptor.

**KIR3DL1 recognizes the Bw4 epitope of HLA-A and HLA-B**

Sequences for >1600 HLA-B allotypes are now known (53), but when first described in the 1960s, HLA-B was a simple serologic dimorphism comprising the 4a and 4b Ags (54), later renamed the Bw4 and Bw6 epitopes, respectively (55). Every HLA-B allotype carries either Bw4 or Bw6, while a subset of HLA-A allotypes carries Bw4. Correlating with the Bw4/Bw6 difference are polymorphic sequence motifs at residues 77–83 in the helix of the HLA class I α1 domain (56), and the capacity for Bw4+ HLA-A and -B allotypes to be ligands for the inhibitory KIR3DL1 NK cell receptor (57, 58), formerly known as NK1B1 (59).

Of the five residues that distinguish Bw4 and Bw6 motifs (77 and 80–83) only arginine 83 is essential for binding KIR3DL1 (60); this contrasts with the position 80 dimorphism specifying the C1 and C2 epitopes recognized by lineage III KIR (61). As an additional important structural difference, lineage III KIR interaction with HLA-C is accomplished with two Ig-like domains (D1 and D2), whereas an additional domain (D0) is necessary for 3DL1 to bind Bw4 (62, 63). Although a crystallographic structure for KIR3D has yet to be achieved, the combination of mutagenesis and homology modeling, based on the three-dimensional structures of KIR2D bound to HLA-C (11), predicts that the D0, D1, and D2 domains contribute equally to the HLA-binding surface in which a central pocket grasps arginine 83 (64). That all human lineage III KIR genes contain an exon encoding D0, but which is no longer used, indicates the more recent evolution of the interaction between HLA-C and lineage III KIR (65).

Some 25–42% of HLA-B and 15–43% of HLA-A allotypes carry the Bw4 epitope (Fig. 3). The Bw4 and Bw6 sequence motifs are frequent targets for short interallelic conversion events. Thus, >51 pairs of HLA-B allotypes differ only in the presence or absence of Bw4. Gene conversion similarly in-

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**FIGURE 2.** KIR3DL1/S1, like HLA-A, -B, -C and HLA-DRB1, is one of the most highly polymorphic human genes. Compared in this figure is coding-sequence diversity in the genomes of two Asian individuals. For the four alleles of each gene, the number of single nucleotide polymorphisms (SNPs) is normalized to the number of codons in the gene. These values are presented in a histogram (yellow bars) and a continuous distribution (blue line). The genes form a normal distribution, with KIR3DL1/S1, HLA-A, -B, and -C, and HLA-DRB1 being outliers. For the named genes, the number of allotypes described worldwide is in parentheses (112, 53). Although KIR3DL2 and KIR3DL3 have many alleles, they differ by one or a few substitutions. Summary statistics are from Wang et al. (113) and Kim et al. (114), and were analyzed using Statistica software version 8 (StatSoft, Tulsa, OK).

**FIGURE 3.** Distribution of HLA-A and -B epitopes recognized by KIR in human populations. Each pie chart represents the HLA-A or -B allotypes in a population. Subdivisions within each pie chart are colored according to the epitope recognized by KIR, or shaded gray if they do not engage KIR. The A3/11 epitope is subdivided into A3 and A11, and the Bw4 epitope is subdivided according to the combination of residues at positions 80 (within the Bw4 motif) and 152, both of which influence the avidity of Bw4 for KIR (115). HLA frequencies for representative Asian (n = 24), African (n = 13) and European (n = 10) populations were obtained from http://www.allele-frequencies.net (116).
introduced Bw4 into HLA-A, where it spread by interallelic conversion to the HLA-A*23, A*24, A*25, A*26, and A*32 allotypes. Although individual allotype frequencies vary between populations, the Bw4 and Bw6 frequencies remain remarkably constant (66), with ~50% of HLA haplotypes providing the Bw4 epitope. This even balance indicates complementary functions for Bw4+ allotypes more focused on T cell immunity and Bw4− allotypes contributing to both NK cell and T cell immunity.

KIR3DL1 and KIR3DS1 segregate as functionally divergent alleles of the KIR3DL1/S1 gene

KIR3DL1 and KIR3DS1 are, respectively, inhibitory and activating receptors that diverge in the domains mediating signal transduction, but have similar ligand-binding Ig-like domains. On the basis of their opposing signaling functions, 3DL1 and 3DS1 were initially considered to be the products of different genes (67), but with segregation studies their allelic relationship was recognized (68) and signified by naming the gene KIR3DL1/S1 and numbering the 3DS1 and 3DL1 variants as a single series of alleles (69). Further complicating the situation, unequal crossing over has produced several KIR haplotypes that either lack 3DL1/S1 (70), have both 3DS1 and 3DL1 (66, 71, 72), or have a fusion of 3DL1/S1 with 3DL2 (73).

Functional difference between 3DL1 and 3DS1 is not restricted to signaling. Whereas KIR3DL1 recognition of Bw4 can be readily detected in assays of binding and NK cell function (57, 58, 64), 3DS1 has no demonstrable interaction with Bw4, or any other HLA class I epitope (74–76). Despite this apparent lack of function, 3DS1 is present at significant frequency in every human population (66, 77). Another difference is in the subtypic variation: 3DL1 being highly polymorphic and 3DS1 conserved. All human populations have a balance between several 3DL1 alleles, even genetically less variable populations such as the Japanese (five alleles) (78) and Yucpa Amerindians (three alleles) (79), whereas 3DS1*013 dominates all populations, except Sub-Saharan Africans, and is the most abundant 3DS1/S1 allele worldwide (66). When the six residues distinguishing the extracellular domains of 3DS1 from 3DL1 were individually introduced into 3DL1, three abrogated interaction with Bw4 while having only minor affects on conformation and cell-surface expression, which is consistent with 3DS1 having been subjected to strong positive selection for losing its avidity for Bw4 (64).

Human KIR haplotypes form two groups, A and B, that differ in gene content, allele content, variability, and disease association (80–82). Both haplotype groups are present in all human populations, often at even frequency, and are maintained by balancing selection (79). KIR3DL1 is characteristic of A haplotypes, which mainly have polymorphic genes encoding inhibitory receptors, whereas 3DS1 is a characteristic B haplotype gene. B haplotypes are enriched for less polymorphic genes encoding activating receptors with either reduced (2DS1) or undetectable (2DS2 and 3DS1) avidity for HLA class I, compared to their inhibitory counterparts (83, 84). Differential KIR and HLA associations with infectious and reproductive disease suggest that the balance between A and B haplotypes might derive from the former favoring resolution of infection and the latter favoring successful reproduction (79, 85).

Balanced polymorphism between three lineages of KIR3DL1/S1 alleles

KIR3DL1/S1 alleles represent three phylogenetic lineages: 3DS1, 005, and 015, that have existed for >3 million years and are present in all modern human populations. KIR3DS1*01301, 3DL1*005, and 3DL1*01502 are considered the prototypical alleles of the 3DS1, 005, and 015 lineages, respectively, because they are the only alleles present in all human populations (Fig. 4). Simulations point to their maintenance by balancing selection, indicating that each receptor lineage makes distinctive, complementary contributions to NK cell biology. The 015 lineage is uniquely diversified in African populations (Fig. 4, green shading) with commensurate reduction of the 005 and 3DS1 lineages, whereas the 3DS1 lineage is highly represented in Amerindians, and the 005 lineage in Caucasians (66).

NK cell killing assays show that the interaction of KIR3DL1 with Bw4+ HLA class I is sensitive to polymorphisms in the Bw4 motif, notably position 80 (57), to polymorphism at positions away from the Bw4 motif that affect peptide binding (60) and to the sequence of the bound peptide (86–88). KIR3DL1 polymorphism also affects specificity for HLA class I, as seen in both cellular (78, 89) and direct-binding assays (90), as well as inferred by disease-associations (91). For example, measurement of binding for five complexes of defined viral peptide and Bw4+ HLA class I to four common KIR3DL1 alleles gave three patterns of reaction and only eight of the 20 possible reactions (Table I) (90), a proportion identical to that seen in an earlier study using cytotoxicity assays (88). 3DL1*015 and 3DL1*007 have identical Ig-like domains and the same narrow reaction pattern, whereas 3DL1*005 has a broader specificity. 3DL1*001 combines the D0 domain of 3DL1*005 with the D1 and D2 domains of 3DL1*015 and also has a broad but distinctive specificity, illustrating the importance of the D0 domain in ligand-binding specificity.

KIR polymorphism was originally observed through its influence on the proportion of NK cells expressing 3DL1 and the amount of 3DL1 on their surfaces (92). For example, five common 3DL1 allotypes in the Japanese population, 3DL1*005 and 3DL1*007 are expressed at low levels, 3DL1*001 at an intermediate level, and 3DL1*020 and 3DL1*01502 at high levels—a hierarchy reflected also in the proportion of NK cells expressing each allele (78). The relative level of 3DS1 expression remains uncertain because it is detected only by weak cross-reactivity with anti-KIR3DL1 Ab (74, 75). Although KIR3DL1 allotypes differ in their capacity to educate NK cells and inhibit NK cell effector function, these differences do not correlate in a simple way with the level of cell-surface expression. Common in Caucasians, Africans, and South Asians (Fig. 4), 3DL1*004 represents an extreme case with a low level of cell-surface expression (93). Inefficient folding causes most of the protein to be retained within the cell, but the small amount reaching the surface can deliver inhibitory signals (94) and may even educate NK cells (95). Substitution at position 86 in the D0 domain is largely responsible for poor folding of 3DL1*004, but also includes a minor contribution from position 182 in D1. Mutagenesis of 3DL1*015 at 40 sites of natural 3DL1/S1 variation showed that the great majority of substitutions had no affect or caused modest decrease in cell-surface abundance (as detected by
suggesting that protein stability is not the only variable causing allele-specific differences in cell-surface expression (64)—another likely source being transcriptional variation.

**Organization and variegated expression of KIR genes**

Transcription is controlled at the level of the entire KIR locus, which has a conserved organizing framework comprising 3DL3 at the centromeric end, 2DL4 and the 3DP1 pseudogene in the center, and 3DL2 at the telomeric end. Regions of variable gene content lie between 3DL3 and 3DP1, and between 2DL4 and 3DL2. The intergenic regions containing the promoters are small (~2 kb) and highly homologous, except for the 13.4-kb region of unique sequence between 3DP1 and 2DL4 (81).

In hematopoietic stem cells, the KIR locus is inaccessible with transcription prevented by dense methylation, particularly of CpG islands in the promoter region (96, 97). The KIR locus opens up for transcription at a late stage in NK cell development, when it generates a repertoire of NK cells expressing diverse combinations of KIR. The expressed KIR genes have hypomethylated promoters, whereas the promoters of the silenced genes are hypermethylated. The characteristic variegated expression of KIR by mature NK cells is thus determined by diverse patterns of promoter methylation (98, 99).
NK cells express each KIR gene in one of three ways (100), exemplified by the three functional framework genes. All NK cells express 2DL4, a subset of NK cells expresses 3DL2, and few NK cells express 3DL3. These differences correlate with promoter sequence variation that affects the binding of transcription factors, for which there are many potential sites (101). In studying the variegated expression of KIR genes, KIR3DL1/S1 has been the major subject for research (100, 102, 103).

The ∼2-kb intergenic region upstream of 3DL1/S1 contains two separate promoters. The proximal promoter (102), between nucleotides -1 and -255, has two nonoverlapping sites, one promoting synthesis of sense mRNA, the other anti-sense mRNA (104, 105). The distal promoter (106), in the middle of the intergenic region ∼1 kb from exon 1, promotes only sense mRNA. As transcription of a 3DL1/S1 allele begins, the distal promoter makes sense mRNA while the proximal promoter can favor either sense or antisense mRNA. If both promoters make sense mRNA the cell commits to long-term expression of the 3DL1/S1 allele. In contrast, if antisense mRNA is made from the proximal promoter, it hybridizes to sense mRNA made from the distal promoter, which prevents transcription and leads to silencing of the 3DL1/S1 allele. The hybrid mRNAs give rise to a 28-bp PIWI-like RNA detectable only in the subset of 3DL1/S1-expressing NK cells (107). In mature 3DL1/S1-expressing NK cells, most transcripts arise from the proximal promoter, but the distal promoter also contributes (108). Consistent with the distal promoter playing a decisive role in NK cell development is its activation by IL-15, a cytokine inducing NK cell differentiation (109).

Table I. KIR3DL1 polymorphism affects specificity for HLA class I

<table>
<thead>
<tr>
<th>HLA Class I Ligand</th>
<th>Allotype Peptide</th>
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A summary of the binding interactions of four KIR3DL1 allotypes with nine complexes of HLA class I and a viral peptide, as determined by Thananchai et al. (90). Boxes shaded green denote significant binding. Under peptide, the amino acid sequence of the peptide is given and below it, in parentheses, the viral pathogen from which it derives; HIV, CMV, EBV, or Dengue virus. The relationship of the D0, D1, and D2 domains for each 3DL1 allotype is shown; cyan domain designations denote identity with 3DL1*005, magenta domain designations denote identity with 3DL1*015.

Conclusions

Variable NK cell receptors bind to the same complexes of peptide and MHC class I as the αβ TCR of CD8 T cells, and with sensitivity to the structural nuances of both peptide and MHC class I allotype. These interactions contribute to the education of NK cells during development and their effector functions when responding to cells compromised by infection or malignancy, or cells from somebody else, as occurs in pregnancy and
transplantation. Within the individual, sets of inherited genes encoding polymorphic MHC class I and variable NK cell receptor cooperate to produce a diverse repertoire of functional NK cells, which gives versatility and specificity to the NK cell response. This individual variability is compounded at the population level, where the number of possible KIR-HLA class I genotypes can exceed the size of the population.

Despite this diversity and versatility, comparative studies have uncovered an unprecedented degree of species specificity showing that individual receptors and entire systems of variable NK cell receptors have limited life spans. Thus Ly49, KIR3DL, KIR3DX, and CD94/NKG2 are all seen as highly variable NK cell receptors, but in different species of placental mammals. Such evolutionary transience in NK cell receptors could arise from the competing demands of immunity and reproduction, bottleneck compromise between the need for MHC class I to serve both NK-cell and TCRs, or from obsolescence, being too specialized at fighting past infection, and being unable to adapt to current threats. Counterparts to the human KIR system of variable NK cell receptors exist only in simian primates, species in which the coevolution of KIR with MHC class I can be reconstructed. The effects of balancing selection are evident, particularly in humans with their distinctive A and B KIR haplotypes and functionally disparate KIR3DL1 and KIR3DS1 alleles. Such striking qualitative differences are consistent with KIR-HLA class I interactions contributing to two essential but very different functions in human biology: immune defense and reproduction.

Disclosures
The authors have no financial conflicts of interest.

References
52. Sharkey, A. M., L. Gardner, S. Hiby, L. Farrell, R. Apps, L. Masters, J. Goodridge,
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