MHC class I receptors on natural killer cells: on with the old and in with the new

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ABSTRACT

The functions of natural killer (NK) cells are controlled by an abundance of activating and inhibitory receptors. Many of these interact with MHC class I molecules. The MHC system also interacts with cytotoxic T-lymphocytes and has been shown to comprise a rapidly evolving family of molecules. This challenges the functional relationship of NK cell receptors with their ligands. Although individual receptors have become subject to species-specific expansions over evolutionary time, the main themes of the NK cell interaction with MHC class I have been preserved. This review details the interaction of NK cell receptors with MHC class I and discusses their unexpectedly rapid evolution.

INTRODUCTION

The innate immune system is ancient. Unlike the more recently evolved adaptive immune system, features of the innate immune system can be recognized in animals predating the jawed fish, from which humans diverged about 400 million years ago [1]. Such evolutionary conservation implies a fundamental biological role and one which has been maintained whilst other immune system components have developed. Natural killer (NK) cells, the topic to which this review is devoted, are one facet of the innate immune system. They have been described in both Xenopus [2] and the jawed fish [3]. Thus NK cells have been involved in the host defence system of vertebrates for hundreds of millions of years [4].

NK cells were defined morphologically as a subset of large granular lymphocytes [5]. Their key demonstrable function was the ability to lyse MHC class I-negative cell lines in vitro without the necessity for prior activation, i.e. 'natural cytotoxicity'. This evokes the model that they are present in the body as a cell type capable of an instantaneous response to intracellular pathogens. However, recent work has implied that they have other more subtle roles. For instance, their cytokine profile is not uniform and varies according to cell-surface phenotype and state of maturation [6,7]. They can differentially secrete either Th1 cytokines (interferon γ (IFNγ), tumour necrosis factor α (TNFα) and granulocyte/macrophage colony-stimulating factor (GM-CSF)) or Th2 cytokines (interleukin-4 and interleukin-13) and thus shape the nature of the adaptive immune response.

In health it appears that NK cells are tolerant to the host and understanding the mechanisms whereby healthy cells are not attacked by autologous NK cells has led to one of the key paradigms that underpins NK cell biology – 'the missing self hypothesis' [8]. Work in this field has provided insights into fundamental features that distinguish them from conventional T- and B-lymphocytes. The latter cells have functions governed overall by a single antigen-specific receptor, whereas no such obvious single receptor dominance is seen in the NK cell receptor repertoire. Rather, they are controlled by many different receptors with both activating and inhibitory functions (Table 1) [9,10]. It appears that the balance between the signals transduced by these receptors

Key words: C-type lectin-like receptors, evolution, killer cell Ig-like receptor, MHC class I, natural killer cells.
Abbreviations: CMV, cytomegalovirus; ITIM, immunoreceptor tyrosine-based inhibitory motif; KARAP, killer cell-activating receptor-associated protein; KIR, killer cell Ig-like receptor; MCMV, murine CMV; MIC, MHC class I chain-related molecule; NK, natural killer; NKC, NK cell complex; NKG2, NK group 2.
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Table 1  A table of activating and inhibitory NK cell receptors

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is the ultimate determinant of the response of the NK cell on encountering a potential target cell [11]. The mechanisms by which these signals are integrated at an intracellular level to determine outcome are the subject of much research. This review will, however, focus on one aspect of NK cell biology: their cell-surface receptors for MHC class I. This receptor/ligand system is a key feature that defines their interaction with autologous cells and its rapid evolution implies a critical role in defence against pathogens.

NK CELLS AND MHC CLASS I

During their initial characterization, it was observed that NK cells kill selected tumour targets. This ability was related to the expression of MHC class I by the target cells. In these experiments, NK cells did not lyse tumour targets that expressed high levels of MHC class I [12]. These in vitro observations paralleled an in vivo phenomenon called hybrid resistance [13]. This is observed when the bone marrow of a parent, homozygous for MHC class I, is transplanted into its F1 offspring. The outcome, if modelled on classical transplantation biology, would be for acceptance of the graft, as the offspring carries all the MHC class I molecules of the parent. However, in these experiments the bone marrow was rejected and this rejection was due to NK cells [14]. Further observations on transfected cells suggested the essential role of MHC class I molecules as key determinants in preventing lysis of cells. These studies were supported by bone marrow transplantation experiments in the mouse [15,16]. The experimental data were synthesized in a model of NK cell function termed ‘the missing self hypothesis’ (Figure 1) [8]. This suggests that NK cells recognize self MHC class I molecules, and the presence of such molecules protects a cell from NK cell attack.

Antibody studies have demonstrated diversity in the cell-surface expression of NK receptors amongst different NK cell clones. The expression of these clonally distributed receptors on NK cell clones has been correlated with their ability to lyse certain MHC class I target cell lines [17,18]. Thus it was shown that not only is there heterogeneity amongst the ability of certain MHC class I molecules to mediate protection against NK cells, but that the receptors on the NK cells determining this phenomenon were clonally distributed within an individual [19]. This observation introduces the concept of an NK cell repertoire in which NK cells with diverse receptor specificities circulate within a single individual.

The observation that murine and human NK cells share a common mechanism of action suggested that both species would probably share a common receptor ligand system for MHC class I recognition. However, the MHC class I of the two species is homologous, not orthologous, and the precise mechanisms of interaction of NK cells with their ligands in the two species have features in common, but also features that are distinct. Thus both species have inhibitory receptors and activating receptors derived from the CD94/NK group 2 (NKG2) families [20–22]. Conversely, although the receptors for polymorphic murine MHC class I are members of the C-type lectin family (Ly49 receptors) [23,24], those for polymorphic human MHC class I are derived from the Ig superfamily of receptors, the killer cell Ig-like receptors (KIRs) [25,26].

KIRs

Structural overview

KIRs form a family of activating and inhibitory receptors based upon the template of extracellular Ig domains, a stem region, a transmembrane domain and an intracytoplasmic tail. Different domains have distinct functional properties. The extracellular Ig domains are the sites of ligand recognition. The cytoplasmic tail of inhibitory KIRs is long and contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which have
Figure 1  Mechanism of hybrid resistance

Two inbred mouse strains homozygous for MHC class I are crossed, giving rise to an F1 offspring (A). When bone marrow from either parent is transplanted into the F1 offspring it is rejected. The mechanism of this rejection is depicted in (B–D). In (B), the F1 bone marrow expresses both maternal and paternal MHC class I molecules. NK cells in the F1 offspring recognize either paternal or maternal MHC class I (the F1 cells express both) via inhibitory receptors. This interaction prevents lysis of the bone marrow. If maternal bone marrow is transplanted into the F1 offspring (C), then NK cells (NK cell type 1), which express an inhibitory receptor only for the paternal MHC class I, are not inhibited from lysing maternal bone marrow. Thus the bone marrow is rejected. A similar situation for NK cells of type 2, which have inhibitory receptors for maternal MHC class I antigens, is encountered when paternal bone marrow is transplanted.

the amino acid sequence Val/Ile-Xaa-Tyr-Xaa-Xaa-Leu (where Xaa is any amino acid) [27,28]. When the tyrosine residue of this motif is phosphorylated, it binds a phosphatase [e.g. Src homology phosphatase-1 (‘SHP-1’)] and transduces an inhibitory signal (Figure 2A) [29,30]. Activating KIRs have short intracytoplasmic tails and transduce activating signals via interaction with an adaptor molecule DAP12/killer cell-activating receptor-associated protein (KARAP) [31,32]. This interaction is determined by the presence of a positively charged amino acid residue in the transmembrane region of KIRs, which mediates binding to a negatively charged residue in the transmembrane region of DAP12/KARAP.

The nomenclature for KIRs is based upon structure [33]. Thus a KIR with two extracellular Ig domains and a short intracytoplasmic tail is named KIR2DS and given a number as a suffix e.g. KIR2DS2 or KIR2DS4. A KIR with three extracellular Ig domains and a long intracytoplasmic tail is named KIR3DL, e.g. KIR3DL1 or KIR3DL2. More recently the KIRs have been given the CD designation of CD158a–k [34].

At present 14 expressed KIRs have been described. In addition, there are two non-expressed pseudogenes [35,36]. The KIRs exhibit structural and functional heterogeneity and, based on sequence analysis, can be grouped into lineages (Figure 3) [37]. Lineage I comprises two-Ig-domain KIRs, with the Ig domains homologous with the membrane distal and membrane proximal domains of three-Ig-domain KIRs [38,39]. This orientation is designated D0D2, i.e. the domain
homologous with the D1 of three-Ig-domain KIRs is absent. Lineage II comprises the three-Ig-domain KIRs with a specificity for classical MHC class I molecules [40,41]. Lineage III contains the majority of the two-Ig-domain KIRs [25,42]. These have domains homologous with the middle and membrane proximal domains of the three-Ig-domain KIRs, i.e. the D1D2 orientation. These comprise the MHC-C-specific KIRs as well as several activating receptors with as yet undefined ligands. It also includes the two pseudogenes (KIR2DP1 and KIR3DP1). The KIR3DL3 gene does not easily fit into any of the lineages described above. This KIR has an undefined specificity and appears not to be expressed in the majority of individuals [43].

The KIR loci are located on chromosome 19q13.4 and form part of the chromosomal region designated the leucocyte receptor cluster ('LRC') [44]. This region contains three framework genes KIR3DL3, KIR2DL4 and KIR3DL2, which appear to be present in nearly all individuals [36,45]. The regions lying between these genes have been the subject of multiple gene duplication and deletion events. This has provided modern day humans with diverse KIR genotypes. Individuals have between 6 and 14 expressed KIR genes [46,47]. The most common Caucasian genotype appears to be the framework genes mentioned previously in combination with KIR2DL1, KIR2DL3, KIR2DS4, KIR3DL1 and the pseudogenes KIR3DP1 and KIR2DP1 (Figure 4) [48]. Thus individuals homozygous for this combination have only one expressed activating KIR (KIR2DS4), whereas other individuals can have as many as six. Thus there is significant genetic diversity at the level of the locus. This diversity is compounded further by allelic variation: in one study of 143 individuals from 34 families, 98 different KIR haplotypes were defined at the molecular level [49]. At present, the functional significance of this diversity is unclear, but one model is that on a population basis it provides for diversity in the NK cell response against pathogens.

MHC class I specificity

Structural and functional studies have provided insights into how KIRs bind MHC class I. Specificities for
Figure 3  Structure, lineage grouping and MHC class I ligands of expressed human KIRs

The extracellular domains (D0, D1 and D2), the stem region (ST), transmembrane (TM) domain and intracytoplasmic tail (CYT) of the various lineages of KIRs are illustrated. (+) in the transmembrane domain indicates a positively charged residue for interaction with KARAP/DAP12, and (●) denotes the ITIMs. For lineage I, KIR2DL4 is illustrated. This has both the charged residue and one ITIM. It differs from the other member of this lineage, KIR2DL5, which has two ITIMs and no transmembrane charged residue.

Figure 4  Genomic organization of the KIR locus

(A) The 'framework genes' KIR3DL3 (centromeric), KIR2DL4, KIR3DP1 and KIR3DL2 (telomeric), which are present in all human genotypes. The regions lying between these genes have been the subject of many genetic recombination events and have a variable KIR content depending on the KIR genotype of the individual. (B and C) Two haplotypes are illustrated based on the genomic sequencing study by Wilson et al. [36]. The 'framework genes' are depicted in boxes with a solid outline and the other KIR genes in boxes with a dashed outline.

HLA-A, -B, -C and -G have been described (Figure 3). KIR3DL2 is thought to bind HLA-A3 and HLA-A11 [41,50], KIR2DL4 recognizes HLA-G [51,52] and KIR2DS4 is thought to interact with HLA-C [53,54]. The mechanisms of these interactions have not been well defined as yet, and are eagerly awaited. The specificity of KIR3DL1 for HLA-B allotypes with the Bw4 serological motif has been clearly demonstrated [40,55]. Overall, however, the molecular details of the KIR–MHC class I interaction have been most clearly defined for the lineage III KIRs with HLA-C.

The interaction of KIRs with HLA-C defines two distinct groups of HLA-C allotypes: C1 and C2 [18,56]. This is based on a dimorphism at residues 77 and 80 on the α1 helix of the MHC class I heavy chain. The C1 allotypes have serine and asparagine residues at these positions and the C2 allotypes have asparagine and lysine residues. These two amino acid changes govern the ligand specificity for KIR: KIR2DL1 and KIR2DS1 recognize C2 allotypes, whereas KIR2DL2, KIR2DL3 and KIR2DS2 interact with the C1 group of allotypes [37]. Thus all HLA-C allotypes possess motifs for interaction with KIRs. Similarly, single amino acid changes in the KIR can dramatically affect binding to MHC class I. The amino acid residue at position 44 determines the MHC class I specificity of these KIRs: KIR2DL1 and KIR2DS1 have a methionine residue at this position and KIR2DL2, KIR2DL3 and KIR2DS2 have a lysine residue. The KIR–MHC interaction is sufficiently labile that a single amino acid change at this position can completely change MHC class I specificity from C1 to C2 or abrogate measurable binding totally [57–59].

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The co-crystal structure of KIR2DL2 and HLA-Cw*0304 has demonstrated that these molecules are held together by a number of charged salt bridges and hydrogen bonds [60]. Perturbation of any one of these salt bridges dramatically diminishes KIR binding. One key hydrogen bond interaction is between Lys44 of KIR2DL2 and Asp50 of HLA-Cw*0304. Overall the co-crystal structure of KIR2DL1 and HLA-Cw*0401 is broadly similar to that of KIR2DL2 and HLA-Cw*0304 [61].

Despite having three extracellular Ig domains, the interaction of KIR3DL1 with its MHC ligands appears similar to that of the MHC-C-specific two-Ig-domain KIRs. KIR3DL1 has been shown to interact specifically with HLA-B allotypes with the Bw4 serological motif [40,62]. This motif is a cluster of seven amino acids (residues 77–83) on the α1 helix of the MHC class I heavy chain that overlaps one of the key binding sites for the MHC-C-specific KIRs. Mutagenesis studies have demonstrated that several MHC I contact residues in the D1 and D2 domains of the KIR3DL1 are similar to those involved in binding KIRs to MHC-C. These include the residue at position 139, which is equivalent to those involved in binding KIRs to MHC-C. Despite the similarity, the interaction of KIR3DL1 with MHC-C appears less avid than for the inhibitory KIRs [64]. In this interaction, D0 domain is required for a functional interaction between KIR3DL1 and HLA-B [64]. In this interaction, D0 domain might function as an enhancer of binding to KIR3DL1 and HLA-B allotypes with the Bw4 serological motif [59,68]. The theme of peptide dependence is shared by both KIR2DL2 and KIR3DL1 and exerts a further constraint on the KIR-MHC class I interaction. Thus there is potential for the KIR-MHC class I interaction to be perturbed by viral infections that do not necessarily down-regulate MHC class I expression, but merely cause a change in the peptide presented.

NKG2 FAMILY OF RECEPTORS

Although KIRs interact with polymorphic MHC-A, -B and -C molecules, another family of receptors controlling NK cell function interact with much less polymorphic MHC class I molecules, the class Ib molecules. These receptors are the NKG2 family of receptors. First cloned in 1991, these genes form part of the NK cell complex (NKC) on chromosome 12p13 [21,69]. These represent five distinct open reading frames: NKG2A, NKG2C, NKG2D, NKG2E and NKG2F and the splice variants NKG2B and NKG2H. Structurally they are members of the C-type lectin-like family of receptors. The basic structure of these molecules is that of an intracellular N-terminal domain, a transmembrane domain and an extracellular carbohydrate-recognition domain, which is the ligand-binding part of the molecule. Similar to the KIRs and on the basis of similar functional motifs, ITIMs or a transmembrane-charged amino acid residue, these receptors can also be divided into activating and inhibitory receptors. Other members of this family involved in NK cell function are the rodent Ly49, NKR-P1 and CD94 [70]. At least four of the NKG2 family of molecules (NKG2A, NKG2C, NKG2D and NKG2E) are thought to represent functional receptors. However, within this grouping NKG2D is structurally divergent, having only 20 % sequence homology with the other three molecules, whereas the NKG2A, NKG2C, and NKG2E receptors have > 94 % sequence homology. Furthermore in order to generate functional receptors NKG2A, NKG2C and NKG2E are required to form heterodimers with the CD94 protein, whereas NKG2D forms functional homodimers (Figure 2B). Finally NKG2D has ligands and signalling pathways distinct from those of the other members of the NKC [71].
The ligand specificity of these receptors has recently been defined. The NKG2A, NKG2C and NKG2E receptors recognize HLA-E [72]. This class I molecule is non-polymorphic, but similar to other MHC class I molecules in that it forms a trimeric complex with β2 microglobulin and a nonamer peptide. In order for it to be stably expressed on the cell surface, it must bind its cognate peptide. This peptide is usually derived from the leader peptide of other MHC class I molecules, but not its own [73]. Such a peptide can be derived from various HLA-A, -B, -C and -G leader sequences. It has been demonstrated that a cytomegalovirus (CMV) protein (UL40) and a heat-shock protein (hsp60) can also furnish the HLA-E-binding peptide [74,75].

Despite its structural divergence from KIRs, the CD94/NKG2 system shares several functional features with the KIR system. Firstly, there are inhibitory (NKG2A) and activating receptors (NKG2C and NKG2E). Secondly the activating receptors bind more weakly to their ligand than the inhibitory receptors. Finally, the binding of the receptors to their ligands is peptide-dependent [76,77].

NKG2D has functional and signalling properties distinct from those of the other NKG2 molecules and has a different repertoire of ligands. The human NKG2D receptor binds to the stress-inducible proteins MHC class I chain-related molecule (MIC)-A and -B and also the UL16-binding proteins ('ULBPs') [78–80]. These molecules share <50 % sequence identity and successful binding to such divergent ligands is accomplished by good shape complementarity at the receptor–ligand interface, differences in the receptor ligand docking orientation and conformational changes in the loops of NKG2D [81]. At a functional level, NKG2D transduces an activating signal via the adaptor proteins DAP10 and DAP12 [82,83]. The interaction with DAP10 is confined to NKG2D, whereas that with DAP12 is shared with NKG2C/E and also with the activating KIRs. The ability of NKG2D to combine with two independent adaptor molecules gives it a unique ability to activate NK cells in a manner not shared by other NK cell-activating receptors.

CLONAL DIVERSITY OF NK CELLS

In both humans and mice, the MHC class I receptors are expressed stochastically in a clonal manner [33,84]. It has been observed by both antibody studies and functional experiments that NK cells form a population of cells with diverse phenotypes. Such diversity is generated by their ability to express different combinations of receptors. The control of expression of KIRs is determined by DNA methylation [85,86]. Both the KIR genes and the host MHC class I play a role in determining the configuration of the repertoire, but how this is achieved is poorly understood. One model is that all NK cells express at least one inhibitory receptor for self MHC class I. This appears to be the case in cultured human NK cell clones, the model being fulfilled by expression of KIRs or CD94/NKG2A. In mice, however, NK cells can be found that do not fulfil this model. Understanding the mechanisms that govern the control of the NK cell repertoire will give insights into the diversity of the NK cell response to pathogens.

EVOLUTIONARY STUDIES

The NK cell receptor–MHC class I interaction is remarkably preserved at a functional level across the species. Rodents and humans are separated by about 100 million years of evolution [87]. Over this time, both receptors and ligands have evolved at varying rates, but the basic themes have been preserved. Such themes include the maintenance of NKG2D function; the relationship of the CD94/NKG2 heterodimeric receptors with non-classical (class Ib) MHC class I molecules; and the presence of inhibitory and activating receptors for polymorphic class Ia MHC class I molecules. On the background of this is a remarkable diversity in their evolutionary trajectories. This appears to be due to the effects of selective pressures on the receptors themselves to maintain functional interactions with their ligands and also to develop novel interactions as novel pathogens are encountered [88]. An additional pressure is placed on these receptors as their ligands, the MHC class I molecules, can have dual functionality. The classical MHC class I molecules have the additional role of presenting pathogenic peptides to T-lymphocytes. Such a role has probably resulted in the rapid evolution of the MHC class I molecules and challenges their interactions with NK cells.

The C-type lectin-like receptors are present in both rodents and primates (Figure 5) [20,89,90]. NKG2D is one of the most conserved of these receptors between mice and humans. There is approx. 60 % sequence homology between the NKG2D molecules of the two species. Their ligands are, however, surprisingly divergent. Human NKG2D binds MIC-A, MIC-B and the UL16-binding proteins, whereas murine NKG2D binds retinoic acid early inducible gene 1 (Rae1) and H60, a minor histocompatibility antigen [91,92]. NKG2D is expressed by all human NK cells and thus NK cells are activated on encounter with ligands for this receptor. The MIC proteins are not expressed at high levels in resting cells and proteins are up-regulated during a cellular stress response thus targeting a cell for lysis [93]. Similarly, the ligands for murine NKG2D are up-regulated during genotoxic stress [94].

The CD94 gene also shares an orthologous relationship in rodents and primates as is demonstrated by phylogenetic analysis of the C-type lectin-like receptors [90]. In this type of analysis performed for five species.
Comparison of the CD94 and NKG2 genes in five species

The individual loci are listed and boxed if they represent a distinct phylogenetic clade. Boxes connected by lines are thought to be orthologous amongst the species.

<table>
<thead>
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<th>Mouse</th>
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Figure 5  Comparison of the CD94 and NKG2 genes in five species
The individual loci are listed and boxed if they represent a distinct phylogenetic clade. Boxes connected by lines are thought to be orthologous amongst the species.

(mouse, rat, rhesus monkey, common chimpanzee and human), the sequences for the CD94 genes are more closely related to each other across species than they are related to other C-type lectin-like receptors within the same species. This is also true for NKG2D, but not the other members of this receptor family. The NKG2A, NKG2C and NKG2E sequences isolated from rodents form a phylogenetic group or clade, distinct from those from primates. Thus, although both orders have C-type lectin-like receptors with activating and inhibitory forms, it is unclear as to whether they are actually the same gene. Conversely, the same analysis when performed on primates alone demonstrates that the NKG2A, NKG2C/E and NKG2 genes all form more tightly grouped clades based on the genes and not the species. Although the sequences appear conserved, it should be noted that humans and common chimpanzees (Pan troglodytes) (species divergence time of 5–7.5 million years) do not share NKG2C genes precisely. The chimpanzee NKG2C locus has duplicated in comparison with the human and there is greater diversity at these loci in the chimpanzee than at the single locus in the human. Despite this evolution in receptors, the principle underlying the ligands of the NKG2A and NKG2C receptors is conserved between humans and rodents. The receptors of both species interact with a non-classical MHC class I molecule that is up-regulated on binding a leader peptide sequence derived from classical class I MHC molecules. In humans and chimpanzees, it is MHC-E and in mice it is Qa-1 [95]. Thus, although the receptors and ligands have evolved, the essential functional relationship has been preserved.

The most dramatic evolution in the NK cell receptor system is seen for the receptors whose ligands are polymorphic (classical) MHC class I molecules. In rodents and humans, these receptors are represented by entirely different gene families. Human NK cells express KIRs, whereas the rodent NK cells have Ly49 receptors [96]. Both families have diverse members with inhibitory and activating types, both interact with the class Ia MHC class I molecules of their species and both have some degree of dependence on the peptide within the groove of the MHC class I molecule. However, although KIRs are members of the Ig superfamily, the Ly49 receptors are C-type lectin-like receptors and their genes are encoded in the NKC adjacent to those of the CD94/NKG2 family of receptors. In humans, the Ly49 receptors are represented by a solitary pseudogene Ly49E [97]. This gene has been inactivated during primate evolution, most probably at some time after the divergence of the human lineage from that of the orang-utan (Pongo pygmaeus) [98]. Three-Ig-domain KIRs have recently been described in rodents, but their functions have yet to be demonstrated [99].

KIR genes have now been characterized in a number of primates, including humans (Homo sapiens), common chimpanzees, pygmy chimpanzees (Pan paniscus), orangutans and rhesus monkeys (Macaca mulatta) [98,100–103]. Compared with the C-type lectin-like receptors, there is a remarkable degree of sequence divergence with few identifiable orthologies. These studies have demonstrated the presence of species-specific expansions of the different lineages of KIR (Figure 6). It is tempting to speculate that these expansions are related to the evolution of the MHC class I molecules, which are orthologous only between humans and chimpanzees.

Of the 16 KIRs found in humans, only four orthologues are readily identifiable in the common chimpanzee: the lineage I KIRs (KIR2DL4 and KIR2DL5), KIR2DS4 and the framework gene KIR3DL3. This diversity is all the more remarkable when it is considered that the MHC class I loci are orthologous between humans and chimpanzees. This is to such an extent that human and chimpanzee MHC-A, -B, -C alleles are more closely related to the products of the orthologous locus in the other species than to the products of the other loci within the species, i.e. a chimpanzee MHC-A allele is more closely related to human MHC-A alleles than to chimpanzee MHC-B or -C alleles [104]. Within this framework the MHC class I has evolved to such an extent that there are no shared alleles between the species. This poses a significant challenge to KIRs to maintain their functional relationship with MHC class I. Evidence of the maintenance of this relationship is found in both the MHC class I alleles and in cross-species functional experiments.

The motifs that determine NK cell reactivity with the MHC-C are conserved between humans and common chimpanzees. Functional experiments have demonstrated that chimpanzee NK cell clones are restricted by the
same dimorphism at residues at positions 77/80 as are human NK cell clones [101]. This specificity is apparent whether expressed in human or chimpanzee MHC class I molecules. This relationship is also apparent for human NK cell clones when tested against chimpanzee MHC class I allotypes. Thus overall the two species appear to have similar MHC-C specificities. However, there are significant differences, consistent with a rapid evolution of the NK cell receptor system. In humans, KIRs that control MHC class I specificity have two Ig domains. In the chimpanzee, one of the MHC-C-specific receptors (Pt-KIR3DL4) has three Ig domains. Thus, even at this crude structural level, evidence of the evolution of these receptors in tandem with the apparent maintenance of their MHC class I specificity is evident. A more subtle observation is that of the antibody reactivities. Although the blocking monoclonal antibody EB6 marks human NK cells with an inhibitory specificity for MHC-C allotypes of the C2 group, it marks chimpanzee NK cell clones with the opposite MHC-C specificity. This indicates that at least some shared determinants have not co-evolved with the MHC specificity of KIRs. As a corollary to these data, none of the monoclonal antibodies raised against the human C1-specific KIRs that were tested reacted with chimpanzee NK cells.

The common chimpanzee does not have a KIR specific for MHC-B, but has one KIR (Pt-KIR3DL1/2) which interacts with MHC-A and MHC-B allotypes of both species. Sequence analysis of chimpanzee MHC class I demonstrates that the Bw4 motif is present in many chimpanzee MHC class I allotypes and, therefore, this finding would not be predicted from consideration solely of those sequences. It has been suggested that MHC-B is rapidly evolving in the face of the challenge of pathogens
and, therefore, this may have tested the relationship of KIRs to MHC-B more than that to MHC-C. A correlate of this rapid evolution is that, although chimpanzee KIRs recognize human MHC-B allotypes well, human KIRs do not recognize chimpanzee MHC-B allotypes.

At a structural level it has been demonstrated that the D0 domain of Pt-KIR3DL1/2 acts as an MHC class I-binding enhancer and that the predominant MHC class I specificity-determining residues are in the D1 and D2 domains [63]. The D0 domain of Pt-KIR3DL1/2 is a stronger enhancer than that of the human receptor KIR3DL1 and this is due to a two amino acid residue deletion specific to the chimpanzee KIR D0 domain. The stronger enhancing effect of the D0 domain allows the binding of the D1 and D2 domains to individual MHC class I allotypes to be weaker in the chimpanzee than for human KIR3DL1. This permits the overall broader specificity observed for Pt-KIR3DL1/2 when compared with KIR3DL1.

All human KIRs of lineage III have two Ig domains but within their gene structure is a ‘pseudoexon’ which previously would have encoded the D0 domain [105]. The sequence changes that have rendered this exon inactive are different for the different KIRs, and thus this genetic event has been tolerated on multiple occasions throughout evolution. The action of the D0 domain as an enhancer of MHC class I binding is consistent with the assertion that loss of this domain may be permitted, but that the MHC class I specificity is preserved. This is the most parsimonious evolutionary explanation for the observation that human KIR2DL1 and chimpanzee KIR3DL1 have similar MHC class I specificities.

One model explaining the observed similarities and differences between humans and chimpanzees is that different modes of selection are acting on the MHC-C–KIR interaction from those acting on the MHC-B–KIR interaction [101]. For human–pathogen interactions that are dominated by the cytotoxic T-lymphocyte response for successful resolution, there is strong selection pressure for current or novel MHC class I variants that successfully present peptides derived from these pathogens to the cytotoxic T-lymphocyte. This has been proposed in studies of HLA-B [106,107]. Under these circumstances, the KIRs evolve in order to ‘catch-up’ with MHC class I evolution and, if the KIRs could not keep pace with the MHC, then new MHC class I specificities would emerge. The MHC-A- and MHC-B-specific KIRs would fit with this model. By contrast, for infections that are dependent upon NK cells for resolution, the selection pressure would be predominantly on the NK cell receptor system. Thus new KIR variants might emerge, although the MHC remained relatively constant. This selection process might drive variants that interacted in slightly different ways with ‘favourable’ MHC class I allotypes or that were more able to recognize specific peptide repertoires. Although these hypotheses remain to be formally tested, this mode of evolution is consistent with the observations of the evolution of MHC-C-specific KIRs.

The evolution of the NK cell receptor system is unexpectedly rapid considering that the innate immune system is relatively ancient. Although structures have not been strictly preserved between humans and mice, there is a conservation of functional relationships: NKG2D is present in both species and interacts with inducible ligands; the CD94/NKG2 systems both interact with class Ia MHC molecules derived from the leader peptides of class Ia MHC molecules; and KIRs and Ly49 receptors both recognize class Ia molecules and have degrees of dependence on the peptide present in the MHC class I groove [24, 108, 109].

CLINICAL STUDIES

The rapid evolution of NK cell receptor systems would suggest that clinical correlates with viral infection would be readily found. However, at present, there is a paucity of studies addressing this particular issue and individuals with pure NK cell deficiencies are infrequently reported. Individuals deficient in NK cells are susceptible to herpes virus infections, including CMV [110]. However, there is no apparent correlation of MHC class I receptors with ability to lyse autologous infected target cells [111]. In murine CMV (MCMV), a more simple relationship has been demonstrated. Control of MCMV infection is genetically determined by the mouse activating receptor Ly49H [112]. This MHC class I receptor binds directly to an MHC class I homologue, m157, which is expressed by MCMV on the surface of infected cells. This is the first illustration of how a viral infection might impose a selective pressure for the presence of an NK cell receptor.

In humans, genetic evidence for involvement of KIRs in viral infections has come from an exhaustive study of HIV-infected patients [113]. In these individuals, the presence of KIR3DS1 in conjunction with potential MHC class I ligands (MHC-B allotypes with Ile85) was associated with a delayed progression to AIDS. This implied that an activating receptor was an important factor in control of infection. This is consistent with the observed diversification of the activating KIRs during human evolution.

Although no other correlations of KIR genetics with the outcome of viral infections have been found, two studies have implicated activating KIRs in the development of the autoimmune disorders psoriatic arthropathy and rheumatoid arthritis [114, 115]. In these diseases, it may be that KIR-expressing T-lymphocytes, and not NK cells, are the critical effector cells [116].

The study of MHC I expression in tumours permits the testing of a simple model of NK cell function,
namely that loss of an MHC class I ligand by selective down-regulation permits lysis of the tumour cell by removal of an inhibitory signal to the NK cell. This has been demonstrated in a murine tumour model in which specific NK cell clonotypes were recruited to the tumour dependent on the MHC class I type of the tumour [117]. In humans, it is not as clear and, although a number of studies have documented selective class I down-regulation on tumours, the role of NK cells in permitting tumour cell growth has not been well characterized. Conversely, tumour-infiltrating T-lymphocytes may express inhibitory receptors and thus prevent the T-cells from lysing the tumour. This has been shown for melanoma for both KIRs and CD94/NKG2A receptors [118,119].

The other clinical scenario readily accessible to the study of NK cell receptors is that of bone marrow transplantation. In these circumstances, individuals can be matched or mismatched for KIRs, but are usually matched for MHC class I. A clear role for KIRs has been hard to define [120,121]. However, individuals given haplo-identical bone marrows for severe life-threatening disease have provided insights into the role of NK cell receptors. These individuals share only three out of six MHC class I molecules. Thus there is much greater potential for NK cell reactivities based on the presence or absence of a specific KIR ligand. In humans and also in mice, individuals with alloactivities based on the NK receptor–MHC class I interaction had a more favourable outcome in terms of both graft versus host disease and graft versus leukaemia effect [122]. This is explained by the fact that donor NK cells kill recipient dendritic cells and also residual tumour. This killing is due the recipient cells not expressing a ligand for the donor inhibitory KIR. Such a ligand is present in the donor and, therefore, donor NK cells ‘educated’ on this background fail to recognize recipient cells as self and thus they are lysed as would be predicted by the ‘missing-self’ model.

CONCLUSION

Expression of MHC class I-specific receptors by NK cells is a functionally conserved evolutionary system. The major themes of the system have been conserved at a functional level. Despite this, the selection pressure placed on this system has driven a rapid evolution of some of the individual components. The precise mechanisms of receptor–ligand interaction have been subject to much evolutionary selection pressure. The end result of such pressure is manifest in modern day humans as the presence of an unpredicted genetic diversity in the NK cell repertoires of individuals. The next challenge is to determine how this diversity impacts on modern day problems and whether it can be harnessed successfully for therapeutic benefit.
References


24 Hanke, T., Takizawa, H., McMahon, C. W. et al. (1999) Immunoreceptor DAP12 bearing a motif of NKB1 are required for negative signaling and for association with protein tyrosine phosphatase 1C. J. Exp. Med. 184, 295–300


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62 Gumperz, J. E., Barber, L. D., Valle, N. M. et al. (1997) Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NK1B killer cell-inhibitory receptor. J. Immunol. 158, 5237–5241


68 Maenaka, K., Juji, T., Nakayama, T. et al. (1999) Killer cell immunoglobulin receptors and T cell receptors bind peptide-major histocompatibility complex class I with distinct thermodynamic and kinetic properties. J. Biol. Chem. 274, 28329–28334


79 Cosman, D., Mullberg, J., Sutherland, C. L. et al. (2001) ULBP’s, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 14, 123–133


