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The genetic and evolutionary balances in human NK cell receptor diversity

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Abstract

In primates and cattle two ancient killer-cell immunoglobulin-like receptor (KIR) lineages independently evolved to become diverse NK cell receptors. In mice, *KIR* genes were sidelined to the X chromosome, a possible consequence of pathogen-mediated selection on the receptor for IgA-Fc. In humans, *KIR* uniquely form two omnipresent haplotype groups (*A* and *B*), postulated to play complementary and necessary roles in immune defense and reproduction. The basis of *KIR3DL1/S1* polymorphism is three ancient lineages maintained by long-term balancing selection and present in all human populations. Conserved and variable NK cell receptors produce structurally diverse NK cell receptor repertoires within a defined range of missing-self response.

1. Introduction

Natural killer (NK) cells are a heterogeneous population of circulating lymphocytes that contribute to the immune response against infection and also to the biology of mammalian reproduction. As was formerly established for CD8 T cells, the interactions of MHC class I molecules with specific lymphocyte receptors are proving to be major mechanisms that influence both the developmental maturation of NK cells and the execution of their effector functions. Comparative studies, of individuals, population and species have shown that some of the interactions between MHC class I and NK cell receptors have been highly conserved while others are diverse and rapidly evolving under natural selection for better defence and reproduction. NK cells achieve diversity through germ-line mutation, contrasting with the somatic mutation used by the T-cell receptors. In this review, which is in part based upon research seminars I gave at Cambridge University during a sabbatical with John Trowsdale and Ashley Moffett in the autumn of 2006, I will consider five topics that have emerged from our studies to understand the structure, function and evolution of NK cell receptors for MHC class I.

The first topic concerns the convergent evolution of structurally different proteins as highly variable NK cell receptors for classical MHC class I molecules. Why do horses follow mice in their use of Ly49 receptors, while the cattle have taken the human option: the killer cell immunoglobulin-like receptors (KIR). The second topic concerns the compact organization of the human leukocyte receptor complex (LRC), which contains genes for the KIR and many factors involved in defense and reproduction. Because of linkage and linkage

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disequilibrium, the selection on one locus can affect the others. In particular, can selection on the gene encoding the Fc receptor for monomeric IgA explain the lack of KIR genes in the LRC of the mouse? The third topic considers the different and potentially conflicting demands of defense and reproduction, and the necessity of both for the survival of mammalian populations and species. Evidence is presented that suggests the model in which the group A KIR haplotypes are more adapted to immune defense and the group B KIR haplotypes are more adapted to reproduction. As far as is known all human populations maintain a balance between the A and B KIR haplotypes, and the same is true for the three allelic lineages of the highly polymorphic *KIR3DL1/S1* locus, the fourth topic discussed. At this locus the most common allele is arguably the one we know least about what it does. In the final topic I discuss recent studies to examine the heterogeneity of human NK cells and how it is influenced by the extraordinary allotypic variation of HLA class I and the KIRs. What can be said about the rules that govern and restrain populations of NK cells.

2. Humans and Cattle expanded different, ancient lineages of KIR

The realization that humans and mice use the structurally unrelated and independently evolved KIR and Ly49 as their highly variable NK cell receptors for polymorphic MHC class I led to the examination of other species of mammal (reviewed in [1]). Another rodent, the rat, was seen to have expanded Ly49, but not KIR, whereas other primates had expanded KIR, but not Ly49. Curiously, horses were seen to have diversified Ly49 [2,3] whereas in cattle it is the KIRs that are diverse [4–6]. Such unexpected affinities begged the question: why in this aspect of immunity were cattle more similar to humans than horses?

The solution to this conundrum awaited the discovery and characterization of a human KIR gene [7] that was overlooked, or mislaid, when the KIR and LILR gene families were first assembled as part of the human gene project [8]. The location of this gene, originally called *KIR3DL0* but subsequently renamed *KIR3DX1*, is ~180kb away from the expanded cluster of human KIR and in between the two clusters of LILR genes. KIR3DX1 is conserved in primates, phylogenetic comparison showing it forms an outgroup to all the other primate KIR. On this basis *KIR3DLX1* was considered to represent the ancestral KIR lineage from which all the other primate and non-primate KIR were derived: hence its original name of *KIR3DL0* – the zeroth KIR [7].

A more extensive phylogenetic analysis, which included the known primate and non-primate KIR, showed they cleanly divided into two lineages [9]. The 3DX lineage comprises primate 3DX1, two elephant KIR and all but one of the cattle KIR. The 3DL lineage comprises all primate KIR bar KIR3DX1, the exceptional cattle KIR, and all the KIR described for mouse, rat, pig, horse and cat. Distinguishing these two KIR lineages is a repetitive element called LTR33A/MLT1D that is present in intron 3 of the 3DL lineage but not the 3DX lineage. The results of this analysis indicated that *KIR3DX1* was not ancestral to all the other KIR and favored a different evolutionary model.

This revised model proposes an ancestral *KIR3D* gene that duplicated to give one daughter KIR gene that was the common ancestor of the 3DX lineage and a second daughter KIR gene that was the common ancestor of the 3DL lineage [9]. In primates the 3DX lineage has been maintained as a single-copy gene, while the 3DL lineage expanded and evolved to high diversity through processes of gene duplication, deletion and conversion. Conversely, in cattle the 3DL lineage was maintained as a single-copy gene, while the 3DX lineage evolved diversity through gene duplication, deletion and conversion. The separation of the 3DL and 3DX lineages is estimated to have occurred 135 +/- 10.5 million years ago, a time long before the mammalian radiation, but after the genesis of the egg-laying mammals (monotremes) and coincident with the emergence of the marsupials. Thus, the two types of

KIR gene– *3DL* and *3DX* – existed for many tens of millions of years before cattle and humans diverged from a common ancestor. After that divergence the cattle expanded one lineage and the primates the other lineage. In answer to our question, the expanded human NK cell receptors are now seen not to be as similar to cattle NK cell receptors as we originally thought; humans, cattle and horses have variable NK cell receptors that all derive from ancient and independent expansions of different progenitor genes.

So far, no mammalian species has been found that expanded both the *3DL* and *3DX* lineages of *KIR*. Likewise, no species has been identified that has expanded both the *KIR* and *Ly49* genes. Neither is it necessary for mammalian survival to have expanded either *KIR* or *Ly49*: in the pig genome both *KIR* and *Ly49* are represented by a single gene [3,10] and ongoing analysis, by John Hammond in my group, of NK-cell receptors in four species of pinniped (seals and sealions) shows they have just one *Ly49* gene and one *KIR* gene, both of which are conserved, non-polymorphic and expressed at cell surfaces (Hammond et al manuscript in preparation).

3. Why have so many mammals lost their Fc receptor for monomeric IgA?

In the leukocyte receptor complex and flanking the *KIR* locus on its telomeric side lies *FCAR*, the gene that encodes Fc α R1 (CD89) the myeloid cell Fc receptor for monomeric IgA (reviewed in [11]). This receptor can play a useful role in the immune response to extracellular infections, particularly those caused by bacteria. Bacteria-specific IgA in plasma coat the bacteria and opsonize them for phagocytosis by macrophages and neutrophils, or for uptake by dendritic cells. When the activities of Fc α R1 were first described in humans the findings were viewed with some skepticism, because nothing like it could be found or detected in mice [12]. These results were reconciled when Fc α R1 was discovered in cattle [13] and rats [14] and the *FCAR* gene was found to be absent from the mouse leukocyte receptor complex and everywhere else in the mouse genome. In this deficiency the mouse is not alone, because the *FCAR* genes in rabbit and dog also appear non-functional [15]. Species comparisons support the view that a functional *FCAR* gene was present in the common ancestor of placental mammals. Thus, humans, rat and cattle retain this ancestral state whereas mice, rabbits and dogs have independently evolved to a derived state in which, through various mechanisms, Fc α R1 function has been lost by inactivation or elimination of *FCAR*. Given that such a small survey identified several mammalian species that lost Fc α R1 function, it seems likely that a significant number of species have, at some time in their history, gone down this evolutionary route. So why does this happen?

Mammalian genomes are heavily invested in immune defense and pathogen genomes are similarly committed to the evasion, subversion and exploitation of the mammalian defenses. In circumstances where a dominant pathogen is neutralizing a host defense mechanism, or using it for nefarious purpose, it can become advantageous or expedient for the host to scuttle the defense. So under what circumstances is Fc α R1 exploited by pathogens?

Common bacterial pathogens like *Streptococcus pyogenes* and *Staphylococcus aureus* evade Fc α R1-mediated capture by secreting proteins that bind to the Fc of IgA and prevent it from being recognized by Fc α R1. The bacteria become coated with IgA but they cannot be bound, phagocytosed and destroyed by myeloid cells expressing Fc α R1. The presence of such bacterial decoys, which compete with Fc α R1 for the attention of IgA-Fc, is expected to pressure the mammalian host and to select for variants of IgA and Fc α R1 that avoid the bacterial decoy. Evidence for such selection is apparent in the sequences of IgA-Fc and IgA from different species [15].

In humans and chimpanzees *FCAR* is a moderately polymorphic gene with variability in the range observed for *KIR2DL* (the *KIR* genes that encode inhibitory HLA-C specific NK-cell

receptors [1]). In higher primates the EC1 domain of Fc α R1, which directly contacts IgA-Fc, has been subject to positive selection that changed and refined the binding site for IgA. In contrast the EC2 domain, which does not contact IgA-Fc, has been subject to purifying selection that preserves the existing sequence. Here is evidence for the selection of new variants of Fc α R1 that bind more tightly to IgA-Fc and compete more effectively with the bacterial decoys [15].

In higher primates, IgA-Fc has also been to positive selection in its Ca2 domain but not in its Ca3 domain. The Ca2 residues that have been subject to positive selection form two clusters. One cluster is near the binding site for Fc α R1 where change could strengthen the binding to Fc α R1 or weaken the binding to bacterial decoys. As the second cluster is far away from the binding site for Fc α R1, it is less likely to contribute interactions with either Fc α R1 or bacterial decoys and thus provides a candidate site for binding to other host or microbial proteins that have yet to be identified.

Independent evidence for selection on IgA-Fc is that the SSL7 decoy protein of *S. aureus* differentially binds to IgA from different mammals: strongly to human, chimpanzee and pig, weakly to horse and rat, and undetectably to mouse and rabbit. These differences correlate with sequence variation at seven positions in IgA-Fc of which five are on the IgA surface near the binding site inferred by the study of mutant IgA, and subsequent to our study confirmed by crystallography [16]. None of the substitutions in IgA that weaken the interaction with SSL7 were present in the IgA of the common mammalian ancestor; these changes are derived, having been selected for the resistance they confer to subversion by bacterial proteins like SSL7.

Because the binding sites for Fc α R1 and SSL7 on IgA-Fc are largely overlapping, substitutions that permit escape from SSL7 also tend to reduce affinity for Fc α R1 and in some cases may lose the functional interaction. Such a change may be advantageous in surviving infection, providing that bacteria opsonized with IgA can be cleared by another mechanism. If substitutions in IgA that prevent interaction with Fc α R1 become fixed in a population then the function of the *FCAR* gene is lost, even though it still makes the same protein that in past times bound earlier forms of IgA with effect. In these circumstances there is no selection pressure to retain functional *FCAR* genes and they become vulnerable to inactivation or deletion, occurring either by chance or through selection on a linked gene. This sequence of events can explain why mice and rabbits have IgA that is resistant to subversion by SSL7 and have either physically lost their *FCAR* genes (mice) or allowed them to degenerate (rabbit) [16]. In the case of the mouse the event that led to the loss of *FCAR* may also have caused the translocation of the *KIR* genes to the X chromosome [17].

4. Do *A* and *B* *KIR* haplotypes play complementary roles in defense and reproduction?

Human *KIR* are encoded by a diverse, compact gene family in which the expression and function of each gene influences the expression and function of each other gene. From the very first analysis of *KIR* diversity in a human panel it was inferred that *KIR* haplotypes naturally divide into two groups: *A* and *B* [18]. The distinction has held up, and its biological basis is reflected in a range of clinical correlations [1,19]. Although the group *A* haplotypes have fixed gene content of seven expressed genes and two pseudogenes, they are diversified through allelic polymorphism of the individual genes. In contrast the group *B* haplotypes have variable gene content embracing several genes and alleles that are not part of the *A* haplotype. These include genes and alleles encoding activating *KIR* that have elusive functions but are structurally related to the well-characterized inhibitory receptors for the C1 and C2 epitopes of HLA-C and the Bw4 epitopes of HLA-A and HLA-B. In general the

complexity of *KIR* haplotypes in other primate species appears less than in humans [20–22], although this is subject to sampling bias, because no other species has been so intensively examined. Among non-human primates, the chimpanzee is arguably the best studied and it also has the advantage of being the closest relative of the human species. Although chimpanzees have a diverse *KIR* system with clearly defined counterparts to the inhibitory MHC class I specific human *KIR* [23], they do not have an expanded set of activating *KIR* genes of the type characterizing the human *B* haplotypes. Thus the evolution of two distinct groups of *KIR* haplotype distinguished by their content of activating *KIR* genes appears to be specific to human evolution.

All human populations studied have both group *A* and *B* *KIR* haplotypes. The frequencies vary with population: in several population they are relatively even, for example Caucasians [18], whereas the Japanese have a predominance of group *A* haplotypes [24] and Aboriginal Australians a predominance of group *B* haplotypes [25]. That no human population has lost a haplotype group, points to the group *A* and *B* haplotypes having distinctive, complementary functions that have combined to improve the capacity for human populations to compete and survive. Amerindians are a case in point [26]. They have had a harrowing history of extended migration through changing environments, population bottlenecks and episodes of selection by infectious disease. In many respects they are genetically less diverse than the populations of Asia, Europe and Africa. Nevertheless, modern Amerindian populations all retain both the *A* and *B* *KIR* haplotypes, an outcome that is unlikely to have occurred by chance [27–29]. Striking is the Yucpa tribe, for which two gene-content *KIR* haplotypes, *A* and one *B*, account for almost the entire population and are at essentially equal frequency. In addition to preserving the *A* and *B* haplotype groups the two dominant Yucpa haplotypes preserve all but one (*KIR2DS3*) of the *KIR* genes found worldwide [27]. Although we know for certain that individual humans who have only *A* or *B* haplotypes are well able to survive and make their way in the world, these observations suggest that when populations have historically lost either the *A* or *B* haplotypes they did not compete well with the populations that retained them. Undoubtedly, such human populations have existed but they either died out or were assimilated into populations having the missing haplotype.

NK cells and their *KIR* contribute to two distinct and necessary functions: firstly, they provide immune defense which allows individuals to survive infections; secondly, they contribute to reproduction which allows populations to survive to the next generation [30]. Epidemiological study of hepatitis C virus infection showed that homozygosity for *KIR2DL3* and its cognate ligand HLA-C1 improved the probability of terminating a primary infection with hepatitis C virus and thus preventing the development of an erosive and potentially cancer-producing chronic infection [31]. *KIR2DL3* is a characteristic of group *A* *KIR* haplotypes whereas its *KIR2DL2* counterpart on group *B* haplotypes does not protect against chronic infection. Here is an example where homozygosity for the *A* haplotype confers advantage in repelling a dangerous infectious disease. Epidemic infections of viruses like hepatitis C are therefore expected to increase the *A* haplotype frequency in an affected population and concomitantly reduce the *B* haplotype frequency. The signature for such an episode of selection is evident in the *KIR* distribution of the Japanese population, for whom *A* *KIR* haplotypes, *KIR2DL3* and HLA-C1 are all at very high frequency [24].

Epidemiological study of pregnancy syndromes has shown that women who are homozygous for *AA* haplotypes are at higher risk than women who have a *B* haplotype [32,33]. The highest risk being for an *AA* mother bearing a fetus of HLA-C2 type. Here is an example where being homozygous for the *A* haplotype is disadvantageous and where *B* haplotypes confer the advantage. Evidence that pregnancy syndromes have been a selective

force in the evolution of human populations is the inverse correlation worldwide between the frequency of *A KIR* haplotypes and HLA-C2 [32].

If these examples have generality they could in a rather simple way explain the complementarity and balance between the group *A* and *B* haplotypes in human populations. They suggest that the *A* haplotypes are more specialized towards immune defence and the *B* haplotypes are more specialized towards reproduction. In this scenario selection by epidemic disease will increase the frequency of *A* haplotypes in a population, because individuals with *B* haplotypes are more likely to succumb from infection than those who are *AA*. Once an epidemic is over and the survivors compete to expand the population, the frequency of *B* haplotypes will increase because *AA* mothers and their babies will be more likely to die from pregnancy syndromes, whereas mothers with *B* haplotypes will be more likely to survive and have multiple offspring. Over the long term the populations that survive through multiple episodes of epidemic disease and fluctuating population size will be those that retain both *A* and *B* haplotypes.

5. Balancing activating and inhibitory allotypes at a highly polymorphic NK-cell receptor locus: *KIR3DL1/S1*

Gene-content diversity of haplotypes can be considered the first level of genetic variation in the *KIR* system. The second level is allelic polymorphism [34]. Here investigation has concentrated on the *KIR3DL1/S1* locus because it is highly polymorphic and the ligands for its receptors are the products of *HLA-A* and *-B* the most polymorphic MHC class I genes identified [35].

KIR3DL1 allotypes are inhibitory receptors that recognize the Bw4 epitopes carried by some *HLA-A* and *HLA-B* allotypes. *KIR3DS1* is an activating receptor with similar extracellular Ig-like domains to *KIR3DL1*, but a divergent signaling domain (transmembrane region and cytoplasmic tail). Despite its similarity in the extracellular ligand-binding domains, direct binding of 3DS1 to Bw4 has yet to be demonstrated [36] although such interaction is inferred from clinical and functional correlations [37,38]. These qualitative and quantitative differences between 3DL1 and 3DS1 in signaling and ligand-binding functions are of particular interest, because in most situations *3DL1* and *3DS1* segregate as alleles. Exceptions to this rule are *KIR* haplotypes produced by unequal crossing over that have both *3DS1* and *3DL1* [39,40].

The difference between inhibitory 3DL1 and activating 3DS1 is the fundamental aspect of *KIR3DL1/S1* variation. This difference originated with a recombination between a 3DL1 allele and a gene encoding an activating *KIR*, which replaced the inhibitory signaling domain of a 3DL1 allotype with the activating signaling domain of this other *KIR* [41]. This seminal event is estimated to have occurred ~7 million years ago, before the separation of human and chimpanzee ancestors. Following this event the ancestral 3DS1 rapidly acquired several amino-acid substitutions in the extracellular domains that distinguish 3DS1 and 3DL1 and account for their different ligand-binding properties. Subsequently, 3DS1 and 3DL1 both acquired additional polymorphisms, but in very different ways [35].

A worldwide survey of human populations showed that 3DS1*013 is dominant, accounting for 97% of all 3DS1 allotypes [35]. Six additional 3DS1 allotypes differ from 3DS1*013 by single substitutions and are present only in one or a few populations. In essence, 3DS1 is homogeneous. Phylogenetic analysis shows this is not the consequence of a recent selective sweep, but has been the situation for much of the time since 3DS1 first emerged. During this same time frame 3DL1 has split into two distinctive lineages, typified by 3DL1*005 and 3DL1*015, which further diversified into many different variants. Only 34% of the detected

3DL1 allotypes are accounted for by the most common allotype, 3DL1*015, and in almost all populations several 3DL1 allotypes are well represented and subjected to balancing selection [24]. Thus, 3DL1 is polymorphic and has impressively diversified during human evolution. A consequence of the contrasting evolution of 3DS1 and 3DL1, is that the most common allotype specified by the *KIR3DL1/S1* locus is 3DS1*013. Sadly, it also remains the allotype we least comprehend [36].

We now see that *KIR3DL1/S1* comprises three lineages of alleles: *3DL1*005-like*, *3DL1*015-like* and *3DS1*, which are present in all human populations. Since their separation > 3 million years ago, these three lineages have been persistently maintained by balancing selection. Emphasizing their importance, only three *KIR3DL1/S1* alleles are common to all modern human populations and they comprise the most frequent allele in each of the three lineages: *3DS1*013*, *3DL1*005*, and *3DL1*015*. These three alleles must have been present in the ancestral African population from which all modern populations derive and they have been maintained by balancing selection throughout the dispersal of humans from Africa to the rest of the world [35]. This retention of complementary forms is analogous to that we observe for the group A and B *KIR* haplotypes (see section 3). In summation, phylogenetic and population studies indicate that *3DS1*013*, *3DL1*005*, and *3DL1*015* have complementary functions that have been beneficial for the survival and propagation of the human species. Although only a minority of individuals express all three *KIR* lineages (those carrying *KIR* haplotypes containing both *3DS1* and *3DL1*[39,40]), the fact that all human populations retain the three lineages indicate that populations which lost a *3DL1/S1* lineage failed to compete with populations that did not. Although all modern human populations retain the three *KIR3DL1/S1* lineages, the situation in sub-Saharan Africans is different from that of other population groups. In sub-Saharan Africans, balancing selection appears to have given way to a directional selection which has elevated the frequency of the 3DL1*1501-like lineage and depressed the frequencies of the 3DS1 and 3DL1*005-like lineages.

So what are the complementary functions of the three *KIR* lineages? Already mentioned above are the differences between 3DL1, an inhibitory receptor for Bw4, and 3DS1, an activating receptor with an inferred but much weaker affinity for Bw4 [37]. Distinguishing 3DL1*005 and 3DL1*015 are first that 3DL1*005 is expressed at lower levels on the cell surface than 3DL1*015, and second, that they show differential reactivity with Bw4⁺ HLA-B allotypes and different associations with disease. 3DL1*1501-like allotypes more effectively combine with HLA-B57 than with HLA-B27 in reducing progression to acquire immunodeficiency syndrome (AIDS) in people infected with human immunodeficiency virus (HIV). In contrast, 3DL1*005-like allotypes work better with HLA-B27 than HLA-B57 [38]. Such observations are evidence that the two lineages of *KIR3DL1* have co-evolved with different groups of Bw4⁺ HLA-B and/or HLA-A allotypes.

Several lines of evidence are consistent with a model in which the D1 and D2 domains of *KIR3DL1/S1* form the binding site for MHC class I while the D0 domain acts as an enhancer to modulate the effective avidity of that interaction [42]. The mechanism of D0-mediated enhancement is not known but has been speculated to involve higher order contacts between different pairs of cognate *KIR*-HLA class I molecules. Further indicating the functional importance of sequence differences between *KIR3DS1/L1* and the related *KIR* in apes (chimpanzee and orangutan) is evidence for the role of natural selection in diversifying these sequences [35]. Polymorphism at five positions in the D0 domain, seven in the D1 domain and nine in the D2 domain is the consequence of strong selection. The five D0 positions form two separated patches on the surface of the D0 domain that provide candidate sites for mediating enhancement.

A good majority (eleven) of the strongly selected sites in the D1 and D2 domains are residues predicted to contact HLA class I and for which substitution has the potential to alter interaction with HLA class I. Among the residues that form the binding site for MHC class I, almost all the signal comes from inter-species variation that distinguishes the human and ape sequences. In contrast, little of the polymorphic variation within human KIR3DL1 is at residues of the binding site that contact HLA class I, and of the residues distinguishing 3DL1 and 3DS1 only position 166 is a contact residue. Substitution of leucine 166 in 3DL1 for arginine 166 in 3DS1 is therefore predicted to be important for the distinctive reactivities of 3DL1 and 3DS1 with Bw4. Furthermore, conservation of the HLA class I-contacting residues of the KIR3DL1 binding site suggests that the functional effects of KIR3DL1 polymorphism are due to indirect effects and other interactions, including ones with the peptides bound by MHC class I. Notably, the substitutions that distinguish the two 3DL1 lineages do not appear to involve HLA-contacting residues of the KIR3DL1 binding site.

6. Human NK cell repertoires combine structural diversity with functional constraint

The contribution and interplay of six inhibitory HLA class I receptors to peripheral blood NK cell repertoires in a panel of 58 human individuals were recently examined [43]. The analysis was particularly incisive for group *A* *KIR* haplotype homozygotes, because in this genetic background the antibodies used to detect the six receptors (NKG2A, LILRB1, KIR2DL1, 2DL2/3, 3DL1 and 3DL2) are completely specific for the defined inhibitory receptors. For individuals having one or two group *B* *KIR* haplotypes, the antibodies reactive with KIR2L1 and KIR2DL2/3 were also reactive with the activating forms of their respective receptors, KIR2DS1 and KIR2DS2. Nevertheless, the same principles appear to pertain to the full spectrum of human NK-cell repertoires.

With six different receptors it is possible to have 64 different NK cell subsets, including hyporesponsive null cells that express none of the six receptors. All these 64 different NK cell populations were detectable in all of the 58 human donors examined. The relative frequencies of the various subsets varied greatly, but in no individual was there any 'forbidden clone'. Thus there is no wholesale deletion of NK cell subsets based upon the combination of inhibitory HLA class I receptors they express. Individuals who had identical *KIR* genotype and the same set of KIR ligands (drawn from HLA-C1, -C2, -A3/11 and -Bw4) can have different NK cell repertoires as defined by the relative frequencies of the 64 subpopulations of cells. Likely, contributions to these differences come from allotypic heterogeneity within these broad groups of ligands [43].

A remarkably steady component in the system of inhibitory HLA class I receptors is the CD94:NKG2A receptor, which produces an identical missing-self response [44] to class I deficient cells in all human individuals. This constancy reflects the lack of polymorphism in the CD94:NKG2A receptor [45] and in the ligands formed by HLA-E's selective binding to peptides derived from the leader peptides of other HLA class I [46,47]. Contrasting with the constancy of CD94:NKG2A are the inhibitory KIR specific for HLA-C1, C2 and Bw4. The missing-self response of NK cells to these receptors is influenced by allelic polymorphism of the *KIR*, the presence of cognate HLA class I ligand during NK cell development and the allotypic heterogeneity within those ligand groups. Some combinations of HLA class I and KIR give missing-self responses that are in excess of that achieved by CD94:NKG2A, such as the HLA-C1 allotype HLA-Cw*07 and KIR2DL3, whereas others are inferior, like HLA-C2 and KIR2DL1*004. The KIR3DL2 and LILRB1 receptors give weak responses that are not influenced by HLA polymorphism. On the basis of this type of analysis, ligands,

receptors, and their combinations are beginning to be grouped into ones that are strong, intermediate and weak in determining the missing-self response [43].

Although the NK-cell co-expression of CD94:NKG2A and KIR is not forbidden it occurs at a frequency that is less than expected from random association, leading to elevated frequencies of NKG2⁺KIR⁻ and NKG2⁻KIR⁺ NK cells [48]. The relative frequencies of these two NK cell subpopulations varies widely between individuals and results in some repertoires being dominated by NKG2A expression, whereas others are dominated by KIR expression. This difference correlates with the number of strong KIR ligands represented in a person's HLA type. Individuals with no strong KIR ligands have NKG2A-dominant repertoires, because in these genotypes CD94:NKG2A provides the strongest missing-self response. Individuals with one strong KIR ligand are more likely to have KIR-dominant repertoires, in which the cognate inhibitory KIR for the strong ligand is used to give NK cells a stronger missing-self response than is possible with CD94:NKG2A. In such individuals the NK cells expressing KIR and CD94:NKG2A can play complementary roles. Most individuals with two strong KIR ligands have NKG2A-dominant repertoires in which KIR expression is relatively suppressed. This pattern indicates that the contribution of the KIR system to the individual's NK-cell repertoire is controlled within a certain range to complement the function of CD94:NKG2A. In this regard, one strong HLA-KIR combination appears to give the optimal KIR contribution. One strong ligand may be good enough for the individual, but at the level of the population there is advantage to individuals having different strong HLA-KIR combinations [43].

Within the human population the sizes of five broad subsets of peripheral blood NK cells vary widely between individuals. These subsets comprise receptor-negative null cells, NKG2A⁺KIR⁻ cells, NKG2A⁺KIR⁺ cells, NK cells expressing a single inhibitor KIR and cells expressing two or more inhibitory KIR. On the basis of these differences the NKG2A-dominant repertoires divide into two groups and the KIR-dominant repertoires can be divided into three groups. Calculation of the sum of the missing-self response for all NK cell subsets within an individual, and comparing these sums for all 58 members of the study panel, showed that the values distribute over a relatively narrow range, again consistent with the view that the magnitude of the KIR contribution to the NK cell response is regulated and constrained within specific limits [43].

This high-resolution analysis of the repertoire of expression of inhibitory HLA class I receptors by unmanipulated, peripheral blood NK cells provides a minimal assessment of NK-cell receptor diversity. The complexity of the repertoires can only expand as allelic polymorphism of the *KIR* is taken into account as well as the differential expression of the many other families of NK cell receptors. What this study also further emphasizes are the complementary functions of CD94:NKG2A and KIR as conserved and variable inhibitory MHC class I receptors. The interaction between CD94:NKG2A and MHC class I is an ancient one that predated the separation of mouse and man [49]. Subsequent concerted, species-specific evolution of the MHC class I gene family disguised the common origin of human HLA-E and mouse H2-Qa1 from gross phylogenetic analysis, which concluded that they had independently evolved the function of binding peptides derived from the leader peptides of MHC class I and presenting them to CD94:NKG2A [50]. Only when phylogenetic trees were made for the functional residues of the binding-site could the ancient commonality be discerned, whereas elsewhere in the molecule it had been obscured or erased by the recombination and conversion of sequence elements that typify the MHC class I gene family [49].

Whereas non-classical HLA-E has been maintained as a conserved single-copy gene, the HLA-A, B and C determinants recognized by KIR are part of a fast-evolving family of

classical MHC class I genes that differs considerably between all but the most related species [22]. Within the hominoids genes orthologous to HLA-A, B and C have been identified, and to some extent in Old World monkeys. With the New World monkeys correspondence is elusive. Within the higher primates the KIR are also encoded by a diverse and fast-evolving gene families. As a consequence the independently segregating KIR and MHC class I polymorphisms must co-evolve in order to maintain their functional interactions. Adding to the complexity of the situation is the independent selective pressures that are exerted on classical MHC class I genes by the CD8 T-cell response. This complexity appears to have reached its greatest in the human species, where the KIR system is markedly more diverse than in other primates. Buffering the instability of the interactions between KIR and MHC class I is the steadiness of the interaction between MHC-E and CD94:KKG2A.

References

1. Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol.* 2005; 5:201–14. [PubMed: 15719024]
2. Takahashi T, Yawata M, Raudsepp T, Lear TL, Chowdhary BP, Antczak DF, et al. Natural killer cell receptors in the horse: evidence for the existence of multiple transcribed *LY49* genes. *Eur J Immunol.* 2004; 34:773–84. [PubMed: 14991607]
3. Gagnier L, Wilhelm BT, Mager DL. *Ly49* genes in non-rodent mammals. *Immunogenetics.* 2003; 55:109–115. [PubMed: 12712264]
4. Storset AK, Slettedal IO, Williams JL, Law A, Disen E. Natural killer cell receptors in cattle: a bovine killer cell immunoglobulin-like receptor multigene family contains members with divergent signaling motifs. *Eur J Immunol.* 2003; 33:980–90. [PubMed: 12672064]
5. McQueen KL, Wilhelm BT, Harden KD, Mager DL. Evolution of NK receptors: a single *Ly49* and multiple KIR genes in the cow. *Eur J Immunol.* 2002; 32:810–7. [PubMed: 11870625]
6. Dobromylskyj M, Ellis S. Complexity in cattle *KIR* genes: transcription and genome analysis. *Immunogen.* 2007; 59:463–72.
7. Sambrook JG, Bashirova A, Andersen H, Piatak M, Vernikos GS, Coggill P, et al. Identification of the ancestral killer immunoglobulin-like receptor gene in primates. *BMC Genomics.* 2006; 7:209. [PubMed: 16911775]
8. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci USA.* 2000; 97:4778–83. [PubMed: 10781084]
9. Guethlein LA, Abi-Rached L, Hammond JA, Parham P. The expanded cattle *KIR* genes are orthologous to the conserved single-copy *KIR3DX1* gene of primates. *Immunogen.* 2007; 59:517–22.
10. Sambrook JG, Sehra H, Coggill P, Humphray S, Palmer S, Simms S, et al. Identification of a single killer immunoglobulin-like receptor (KIR) gene in the porcine leukocyte receptor complex on chromosome 6q. *Immunogenetics.* 2006; 58:481–6. [PubMed: 16738944]
11. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. *J Pathol.* 2006; 208:270–82. [PubMed: 16362985]
12. Morton HC, Brandtzaeg P. The human myeloid IgA Fc receptor. *Arch Immunol Ther Exp (Warsz).* 2001; 49:217–19. [PubMed: 11478396]
13. Morton HC. IgA Fc receptors in cattle and horses. *Vet Immunol Immunopathol.* 2005; 108:139–43. [PubMed: 16098605]
14. Maruoka T, Nagata T, Kasahara M. Identification of the rat IgA Fc receptor encoded in the leukocyte receptor complex. *Immunogenetics.* 2004; 55:712–6. [PubMed: 14647992]
15. Abi-Rached L, Dorighi K, Norman PJ, Yawata M, Parham P. Episodes of natural selection shaped the interactions of IgA-Fc with FcαRI and bacterial decoy proteins. *J Immunol.* 2007; 178:7943–54. [PubMed: 17548632]
16. Ramsland PA, Willoughby N, Trist HM, Farrugia W, Hogarth PM, Fraser JD, et al. Structural basis for evasion of IgA immunity by *Staphylococcus aureus* revealed in the complex of SSL7 with Fc of human IgA1. *Proc Natl Acad Sci USA.* 2007; 104:15051–6. [PubMed: 17848512]

17. Welch AY, Kasahara M, Spain LM. Identification of the mouse killer immunoglobulin-like receptor-like (Kirl) gene family mapping to chromosome X. *Immunogenetics*. 2003; 54:782–790. [PubMed: 12618911]
18. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, Tyan D, Lanier LL, Parham P. Human diversity in killer cell inhibitory receptor genes. *Immunity*. 1997; 7:753–63. [PubMed: 9430221]
19. Carrington M, Martin MP. The impact of variation at the KIR gene cluster on human disease. *Curr Top Microbiol Immunol*. 2006; 298:225–57. [PubMed: 16329188]
20. Rajalingam R, Hong M, Adams EJ, Shum BP, Guethlein LA, Parham P. Short KIR haplotypes in pygmy chimpanzee (Bonobo) resemble the conserved framework of diverse human KIR haplotypes. *J Exp Med*. 2001; 193:135–46. [PubMed: 11136827]
21. Sambrook JG, Bashirova A, Palmer S, Sims S, Trowsdale J, Abi-Rached L, et al. Single haplotype analysis demonstrates rapid evolution of the killer immunoglobulin-like receptor (KIR) loci in primates. *Genome Res*. 2005; 15:25–35. [PubMed: 15632087]
22. Guethlein LA, Older Aguilar AM, Abi-Rached L, Parham P. Evolution of killer cell Ig-like receptor (KIR) genes: definition of an orangutan KIR haplotype reveals expansion of lineage III KIR associated with the emergence of MHC-C. *J Immunol*. 2007; 179:491–504. [PubMed: 17579070]
23. Khakoo SI, Rajalingam R, Shum BP, Weidenbach K, Flodin L, Muir DG, et al. Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans. *Immunity*. 2000; 12:687–98. [PubMed: 10894168]
24. Yawata M, Yawata N, Draghi M, Little A-M, Parheniou F, Parham P. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *J Exp Med*. 2006; 203:633–45. [PubMed: 16533882]
25. Toneva M, Lepage V, Lafay G, Dulphy N, Busson M, Lester S, et al. Genomic diversity of natural killer cell receptor genes in three populations. *Tissue Antigens*. 2001; 57:358–62. [PubMed: 11380947]
26. Hey J. On the number of New World founders: a population genetic portrait of the peopling of the Americas. *PLoS Biol*. 2005; 3:e193. [PubMed: 15898833]
27. Gendzekhadze K, Norman PJ, Abi-Rached L, Layrisse Z, Parham P. High KIR diversity in Amerindians is maintained using few gene-content haplotypes. *Immunogenetics*. 2006; 58:474–80. [PubMed: 16738943]
28. Ewerton PD, Leite Mde M, Magalhães M, Sena L, Melo dos Santos EJ. Amazonian Amerindians exhibit high variability of KIR profiles. *Immunogenetics*. 2007; 59:625–30. [PubMed: 17551723]
29. Flores AC, Marcos CY, Paladino N, Capucchio M, Theiler G, Arruvito L, et al. KIR genes polymorphism in Argentinean Caucoid and Amerindian populations. *Tissue Antigens*. 2007; 69:568–76. [PubMed: 17498266]
30. Moffett A, Loke C. Immunology of placentation in eutherian mammals. *Nat Rev Immunol*. 2006; 6:584–94. [PubMed: 16868549]
31. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science*. 2004; 305:872–4. [PubMed: 15297676]
32. Hiby S, Walker JJ, O'Shaughnessey KM, Redman CWG, Carrington M, Moffett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of pre-eclampsia and reproductive success. *J Exp Med*. 2004; 200:957–65. [PubMed: 15477349]
33. Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum Reprod*. 2008; 23:972–6. [PubMed: 18263639]
34. Shilling HG, Guethlein LA, Cheng NW, Gardiner CM, Rodriguez R, Tyan D, et al. Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype. *J Immunol*. 2002; 168:2307–15. [PubMed: 11859120]
35. Norman PJ, Abi-Rached L, Gendzekhadze K, Korbel D, Gleimer M, Rowley D, et al. Unusual selection on the KIR3DL1/S1 natural killer cell receptor in Africans. *Nat Genet*. 2007; 39:1092–9. [PubMed: 17694054]

36. Gillespie GM, Bashirova A, Dong T, McVicar DW, Rowland-Jones SL, Carrington M. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8⁺ T cell epitopes. *AIDS Res Hum Retroviruses*. 2007; 23:451–5. [PubMed: 17411378]
37. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet*. 2002; 31:429–34. [PubMed: 12134147]
38. Martin MP, Qi X, Yamada E, Martin JN, Pereyra F, Colombo, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet*. 2007; 39:733–40. [PubMed: 17496894]
39. Martin MP, Bashirova A, Traherne J, Trowsdale J, Carrington M. Expansion of the KIR locus by unequal crossing over. *J Immunol*. 2003; 171:2192–5. [PubMed: 12928362]
40. Gómez-Lozano N, Estefanía E, Williams F, Halfpenny I, Middleton D, Solís R, et al. The silent KIR3DP1 gene (CD158c) is transcribed and might encode a secreted receptor in a minority of humans, in whom the KIR3DP1, KIR2DL4 and KIR3DL1/KIR3DS1 genes are duplicated. *Eur J Immunol*. 2005; 35:16–24. [PubMed: 15580659]
41. Abi-Rached L, Parham P. Natural selection drives recurrent formation of activating killer cell immunoglobulin-like receptor and Ly49 from inhibitory homologues. *J Exp Med*. 2005; 201:1319–32. [PubMed: 15837816]
42. Khakoo SI, Geller R, Shin S, Jenkins JA, Parham P. The D0 domain of KIR3D acts as a major histocompatibility complex class I binding enhancer. *J Exp Med*. 2002; 196:911–21. [PubMed: 12370253]
43. Yawata M, Yawata N, Draghi M, Partheniou F, Little A-M, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires towards a balance of missing-self response. *Blood*. 2008; 112:1437–47. [PubMed: 18425103]
44. Kärre K. Natural killer cell recognition of missing self. *Nat Immunol*. 2008; 9:477–80. [PubMed: 18425103]
45. Shum BP, Flodin LR, Muir DG, Rajalingam R, Khakoo SI, Cleland S, et al. Conservation and variation in human and common chimpanzee *CD94* and *NKG2* genes. *J Immunol*. 2002; 168:240–52. [PubMed: 11751968]
46. Petrie EJ, Clements CS, Lin J, Sullivan LC, Johnson D, Huyton T, et al. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J Exp Med*. 2005; 205:725–35. [PubMed: 18332182]
47. Kaiser BK, Pizarro JC, Kerns J, Strong RK. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc Natl Acad Sci USA*. 2008; 105:6696–701. [PubMed: 18448674]
48. Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, Tyan D, et al. Genetic control of human NK cell repertoire. *J Immunol*. 2002; 169:239–47. [PubMed: 12077250]
49. Joly E, Rouillon V. The orthology of HLA-E and H-2Qa1 is hidden by their concerted evolution with other MHC class I molecules. *Biol Direct*. 2006; 1:2. [PubMed: 16542007]
50. Yeager M, Kumar S, Hughes AL. Sequence convergence in the peptide-binding region of primate and rodent MHC class Ib molecules. *Mol Biol Evol*. 1997; 14:1035–41. [PubMed: 9335143]