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Genetics of Natural Killer Cells in Human Health, Disease, and Survival

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Abstract

Natural killer (NK) cells have vital functions in human immunity and reproduction. In the innate and adaptive immune responses to infection, particularly by viruses, NK cells respond by secreting inflammatory cytokines and killing infected cells. In reproduction, NK cells are critical for genesis of the placenta, the organ that controls the supply of oxygen and nutrients to the growing fetus. Controlling NK cell functions are interactions of HLA class I with inhibitory NK cell receptors. First evolved was the conserved interaction of HLA-E with CD94:NKG2A; later established were diverse interactions of HLA-A, -B, and -C with killer cell immunoglobulin-like receptors. Characterizing the latter interactions is rapid evolution, which distinguishes human populations and all species of higher primate. Driving this evolution are the different and competing selections imposed by pathogens on NK cell–mediated immunity and by the constraints of human reproduction on NK cell–mediated placentation. Promoting rapid evolution is independent segregation of polymorphic receptors and ligands throughout human populations.

INTRODUCTION

Natural killer (NK) cells are a versatile subpopulation of lymphocytes. They make essential contributions to human innate and adaptive immune responses against infection (1), as well as to embryo implantation and establishment of the placenta during a formative stage of human reproduction (2, 3). The breadth of their portfolio shows how NK cells have been instrumental to the survival of human individuals and to propagation of human species ever since *Homo* originated in Africa 2–3 million years ago (Mya) (4). A key feature in NK cell development and in the functional interactions of mature NK cells with sick and healthy cells of the human body are inhibitory NK cell receptors that specifically recognize major histocompatibility complex (MHC) class I molecules. These ligand-receptor interactions educate NK cells to respect the integrity of healthy cells while directing their attack at any cell compromised by infection, malignancy, or other form of stress (5, 6).

NK cells were first named as such in the 1970s, from studies describing mouse lymphocytes that could kill leukemia cells in cultures of one to four hours without "any conventional in vitro induction of immune response" (7; 8, p. 112). In the same decade, human lymphocytes with similar capacity to lyse tumor cells spontaneously in vitro were reported (9). During the 1980s increasing numbers of immunologists were drawn to the nascent discipline of NK cell biology. An attraction of NK cells was the ease and speed of working with lymphocytes that needed no priming. Also attractive was the belief that, "In contrast to cytotoxic T lymphocytes, the recognition of target cells is not dependent or restricted by the major histocompatibility complex" (10, p. 348). This concept had particular appeal to human immunologists, because if HLA diversity could be ignored it would simplify experimental design and expand the potential for applying NK cells to the treatment of patients with cancer (11, 12).

However, as the NK cell field matured, increasing evidence emerged for the involvement of MHC class I, particularly in stimulating NK cell–mediated alloreactions in transplant recipients (13, 14). By drawing a parallel with the cytotoxic T cell, which activates upon recognition of altered self MHC class I, the interpretation of results obtained in NK cell experiments became increasingly complicated, confusing, and unsatisfactory. A clearer picture emerged when experimental data were interpreted using a model in which NK cells respond to reduction or absence of MHC class I on target cell surfaces (15, 16). In other words, NK cells recognize missing self MHC class I (17). To mediate this function, NK cells were postulated to express inhibitory receptors for self MHC class I that prevent NK cells from attacking autologous cells with a normal cell surface level of MHC class I. When MHC class I expression is impaired or lost, as is often the case for infected (18) or malignant (19) cells, inhibitory signaling goes down, leading to NK cell activation mediated by various activating NK cell receptors, including NKG2D (20). Amino acid sequences for the Ly49 receptors of the mouse model were defined in 1989 (21), three years before their function as inhibitory MHC class I receptors was uncovered (22) and explored (23–25). Conversely, functions for the human killer cell immunoglobulin-like receptors (KIR) as MHC class I receptors were appreciated (26, 27) prior to their molecular definition in 1995 (28, 29).

THREE GENOMIC COMPLEXES CONTROL NK CELL EDUCATION AND RESPONSE

In placental mammals, the interactions of MHC class I with NK cell receptors are determined by three genomic complexes, each segregating on a different chromosome (30). In addition to *MHC*, which encodes the class I ligands (31), the natural killer complex (*NKC*) encodes NK cell receptors with lectin-like structures (32, 33), and the leukocyte receptor complex (*LRC*) encodes NK cell

receptors with immunoglobulin-like structures (34, 35). The human *MHC*, the *HLA* region, is on human chromosome 6, the *NKC* is on chromosome 12, and the *LRC* is on chromosome 19. Present in all three complexes are several families of closely related receptor genes, which by asymmetric recombination can readily expand or contract in size. As a consequence of this propensity, there are numerous differences among mammalian species in the number and nature of their *MHC*, *NKC*, and *LRC* genes.

Within the mammalian spectrum, the human family of *MHC class I* genes is small, consisting only of 6 *HLA class I* genes (36). It is also unusually tidy; all 6 genes are fixed in the genome. This is not so for many species. For example, the rhesus macaque has a large family of *MHC class I* genes, including 7 related to *HLA-A* and 19 related to *HLA-B*. Rhesus *MHC* haplotypes differ in length and contain larger, more variable numbers of *MHC class I* genes than *HLA* haplotypes, and none of the genes is fixed (37). The *HLA* region also strikes a balance between three highly polymorphic genes, *HLA-A*, *-B*, and *-C*, and three conserved genes, *HLA-E*, *-F*, and *-G*. All 6 HLA class I isotypes are ligands for one or more of the NK cell receptors (**Figure 1**).

HLA-E, the oldest HLA class I isotype, is the ligand for CD94:NKG2A, the inhibitory receptor that educates the greatest number of human NK cells (38, 39). The genes encoding the lectin-like CD94 and NKG2A subunits of this heterodimeric receptor are in the *NKC*. The mouse has an orthologous interaction between the CD94:NKG2A receptor and Qa1, a conserved mouse MHC class I molecule (40, 41). Such equivalence shows that this particular ligand-receptor interaction existed in a common ancestor of primates and rodents, some 80 Mya, before the radiation of mammalian species (42). It is therefore likely that most, if not all, placental mammals have orthologs of CD94:NKG2A and Qa1/HLA-E.

Other inhibitory NK cell receptors that educate human NK cells are members of the KIR family, which is encoded by a family of *LRC* genes (30). The ligands for KIR are four epitopes of HLA-A, -B, and -C: A3/11, Bw4, C1, and C2. Because of the way these epitopes are distributed among HLA-A, -B, and -C allotypes, an individual can have 1, 2, 3, or all 4 of the epitopes. Whereas all individuals have NK cells educated by HLA-E, the extent to which a person's NK cells are educated by KIR is highly variable and dependent on the individual HLA-A, -B, and -C type (5, 43–47) (**Figure 2**). We thus see how the older, conserved school of NK cell education, mediated by interactions of CD94:NKG2A with HLA-E, is complemented by the younger, more diverse school, mediated by interactions of KIR with HLA-A, -B, and -C. Flanking the *KIR* gene family is the family of leukocyte immunoglobulin-like receptor (LILR) genes (48, 49). These include *LILRB1* and *LILRB2*, which are expressed by NK cells and encode inhibitory receptors with broad HLA class I specificity (50, 51).

The *KIR* locus was deleted from the mouse *LRC*, along with the flanking *FCAR* gene that encodes an IgA receptor (35, 52). Although two *KIR* genes are present elsewhere in the mouse genome (53), they are of unknown function and unlikely to encode NK cell receptors. Instead, the mouse Ly49 family of NK cell receptors recognizes polymorphic MHC class I epitopes. These mouse receptors are functionally analogous to human KIR in many ways, including their expression, diversity, and signaling (23, 54, 55). The human *NKC* contains one *Ly49* gene, which is not functional (56). Being lectin-like receptors encoded by *NKC* genes, mouse Ly49 receptors have no structural similarity to the KIR, and they bind to a site on MHC class I (57) that is completely different from that recognized by KIR (58, 59). From such divergence it is clear that mouse Ly49 and human KIR evolved independently to become variable NK cell receptors for MHC class I but did so in a remarkably convergent manner, presumably under similar selection pressures on NK cell functions.

Surveying mammalian genomes shows how multigene families of *KIR* and *Ly49* genes are restricted to surprisingly few species (**Figure 3**). *KIR* gene families are present in two branches of

a

	HLA class I genes										
	Classical			Nonclassical			Inactivated				
Gene	*A*	*B*	*C*	*E*	*F*	*G*	*H*	*J*	*K*	*L*	*Y*
Alleles	3,968	4,828	3,579	26	25	56	12	9	6	5	3
Allotypes	2,781	3,501	2,490	8	5	18					
Total alleles		12,375			107				35		
Total allotypes		8,772			31						

b

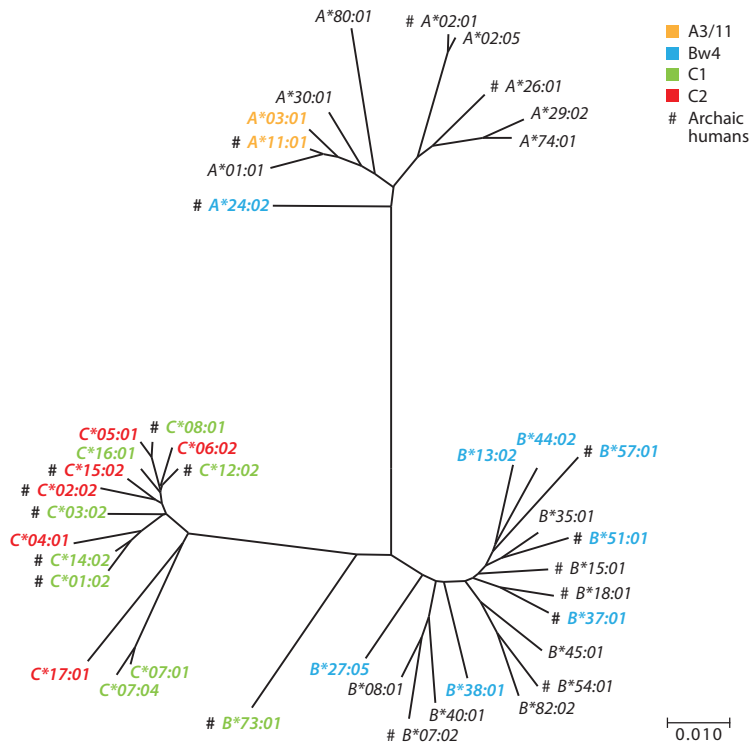


Figure 1

Diversity of *HLA class I* genes and alleles. Panel *a* shows the number of alleles defined for the classical, nonclassical, and inactivated *HLA class I* genes. In addition, there are six *HLA class I* gene fragments: *HLA-P*, *-T*, *-U*, *-V*, *-W*, and *-X*. In the last 15 years clinical *HLA class I* typing has increasingly been performed by nucleotide sequencing of exons 2 and 3, which encode the highly polymorphic α_1 and α_2 domains of HLA-A, -B, and -C. For this purpose, Sanger sequencing is now being replaced by a variety of next-generation sequencing methods (190–193). Several millions of prospective hematopoietic stem cell donors have been typed, and in the process thousands of novel alleles have been uncovered. It is currently estimated that 2–3 million alleles for *HLA-A*, *-B*, and *-C* are present in the human population (139, 194). Most of these alleles are very rare and differ by a single nucleotide substitution from a common allele. Some 1,500 alleles differ by multiple substitutions. This diversity is completely represented by 42 core alleles (11 *HLA-A*, 17 *HLA-B*, and 14 *HLA-C*) (139). Panel *b* shows a phylogenetic tree of the core alleles. Overrepresented in the core alleles are alleles that derive from archaic humans (*hash tags*) and that entered the modern human population by introgression (132).

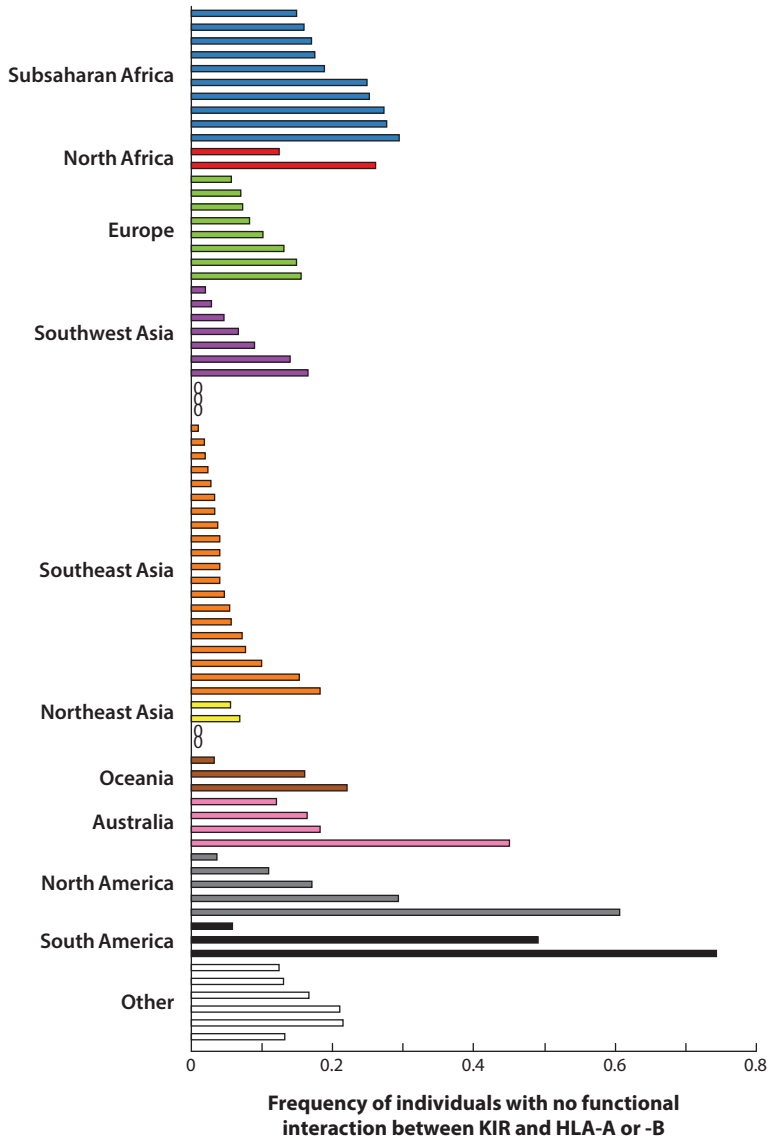


Figure 2

Worldwide frequencies of individuals lacking HLA-A and -B ligands for killer cell immunoglobulin-like receptors (KIR). Genotype data were obtained from dbMHC (**https://www.ncbi.nlm.nih.gov**) and come from 74 populations worldwide and were collected for anthropologic study of *HLA* allele frequencies. Within each population individual genotypes were scored for the presence or absence of alleles encoding HLA-A and -B that can function as KIR ligands, and a population frequency for individuals lacking HLA-A and -B KIR ligands was determined. These values are plotted as the bars of the histogram, which are grouped and colored by geographic region. The "Other" group comprises potentially admixed populations. Values within each geographic region were sorted from lowest to highest. A zero indicates that all genotypes in that population have at least one KIR ligand carried by HLA-A or -B.

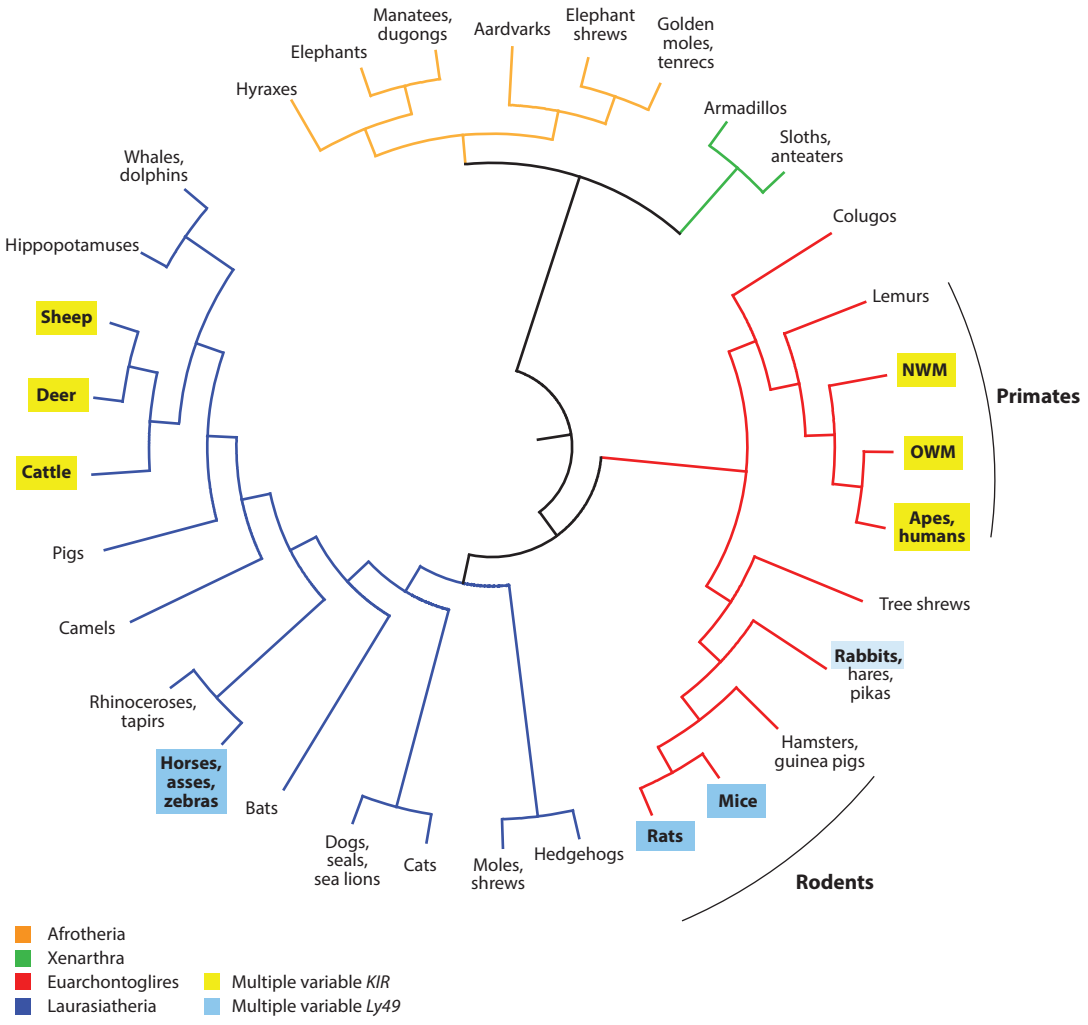


Figure 3

Variable *KIR* and *Ly49* are found in few mammalian species. Shown is a phylogenetic tree of mammalian species based on that of Meredith et al. (42). Branch coloring denotes the four mammalian superorders. Representative species for all the mammalian orders are included. Yellow boxes highlight species with multiple variable *KIR*, blue boxes highlight species with multiple variable *Ly49*. The paler shading of the box for rabbits indicates there are multiple *KIR* in the rabbit genome, though fewer than in mice and rats, but their degree of variability has not been determined. Abbreviations: NWM, New World monkeys; OWM, Old World monkeys.

the phylogenetic tree: simian primates (humans, apes, and monkeys) and ruminants (cattle, sheep, and deer) (60). The primate and the ruminant *KIR* gene families represent independent expansions from different founder *KIR* genes that existed prior to the emergence of placental mammals. Thus the ancestral gene of all cattle *KIR* genes is a pseudogene in the human *LRC* (61). The *Ly49* gene families are also found in two branches of the phylogenetic tree (62): rodents, but not all of them, and equids (horses, asses, and zebras). The nature and extent of these species differences mean that the most informative perspective on the human system of MHC class I ligands and KIR is likely to emerge from its comparison with counterparts in other simian primates.

EMERGENCE OF *MHC-C* CAUSED A REFORMATION OF THE HOMINID *KIR* LOCUS

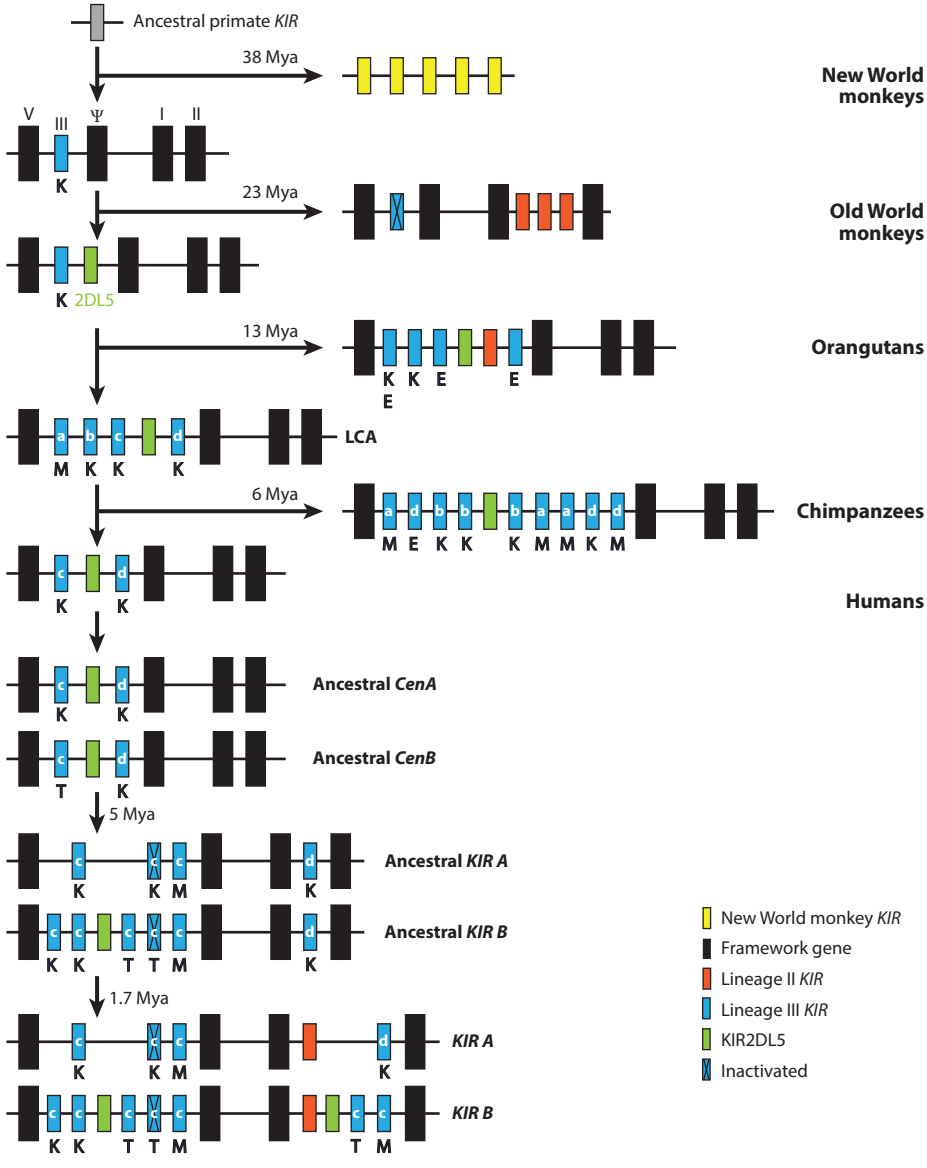
MHC class I and *KIR* of several species of Old (37, 63–65) and New World monkeys have been characterized (66–72) (**Figure 3**). The *MHC class I* and *KIR* of Old World monkeys have much in common with their human counterparts, but not so for those of New World monkeys, in which these ligands and receptors are divergent (67). Counterparts of human *HLA-A* and *-B* are present in Old World monkeys, as are several variants of the Bw4 epitope (73). The human *KIR* gene family, also called the *KIR* locus, and that of Old World monkeys, as exemplified by rhesus macaques, are similarly organized around four framework genes, each representing a different phylogenetic lineage of *KIR* (48, 74). These comprise a lineage V *KIR* at the centromeric end of the locus (*KIR3DL3* in human), followed by the combination of a pseudogene (*KIR3DP1* in human) and a lineage I *KIR* (*KIR2DL4* in human) within the locus and a lineage II *KIR* at the telomeric end of the locus (*KIR3DL2* in human). With some exceptions (75), this framework is a conserved feature of human *KIR* haplotypes (**Figure 4**).

The human KIR that recognize the A3/11 epitope of HLA-A*03 and HLA-A*11 allotypes (76, 77) and the Bw4 epitope (78, 79) carried by subsets of HLA-A and -B allotypes are KIR3DL2 and KIR3DL1, respectively. These human lineage II KIR are encoded by genes in the telomeric region of the *KIR* locus. The corresponding region of the rhesus macaque *KIR* locus contains 19 different lineage II *KIR* genes, with individual haplotypes having between 4 and 15 of these genes (80–82). Thus individual macaques have many more lineage II *KIR* genes than individual humans, as is also true for *MHC-A* and *-B* (83–86). Functional analysis of interactions between rhesus KIR and MHC class I has identified various MHC-A and MHC-B epitopes, including Bw4, that are recognized by rhesus KIR (73, 87, 88). As in humans, these different KIR specificities correlate with polymorphisms in the carboxy-terminal part of the α_1 helix. Overall, the Old World monkey system of interactions between lineage II KIR and MHC-A and -B appears considerably more diverse than that of humans.

One key factor that humans have, but Old World monkeys lack, is MHC-C (89). The human receptors that recognize the C1 and C2 epitopes of HLA-C are the lineage III KIR (90). In the macaque *KIR* locus this lineage is represented only by a pseudogene (91, 92). In phylogeny, the *MHC-C* gene is first seen in orangutans. In these species of Asian great apes, *MHC-C* is not fixed, as in humans, but is a component of ~50% of orangutan *MHC* haplotypes (93, 94). A further difference is that orangutan MHC-C allotypes all carry the C1 epitope, whereas HLA-C allotypes can carry either the C1 epitope or the C2 epitope. Determining the C1/C2 difference is dimorphism at position 80 in the α_1 helix, where asparagine defines C1 and lysine defines C2 (95). As a consequence of *MHC-C*, the orangutan *KIR* locus is impressively different from that of Old World monkeys (74, 96). Gone is the rich diversity of lineage II *KIR* genes (**Figure 4**). The orangutan has only two lineage II *KIR* genes; one is in the centromeric region, and the other is the framework gene at the telomeric end of the locus. New additions to the centromeric region are *KIR2DL5*, a lineage I *KIR* gene of unknown function (97), and four lineage III *KIR* genes. The latter encode inhibitory and activating receptors with high and low avidity for C1 (98). Thus the emergence of *MHC-C* was accompanied by major change in the gene content of the *KIR* locus.

Polymorphism at position 44 in the D1 domain of lineage III KIR determines their specificity for MHC-C and also affects their avidity (99). Orangutan KIR that have lysine 44 (K44) are specific for C1 and bind to it with high avidity (98). In contrast, orangutan KIR that have glutamate 44 (E44) exhibit low avidity for C1 but can also recognize C2 with similarly low avidity (98). Because orangutans lack C2, the physiological difference between the K44 and E44 KIR is one of affinity, not specificity. However, the inherent cross-reactivity of these E44 KIR with C2 provides a

plausible explanation for how the C2 epitope and its cognate receptors first evolved. Converting C1$^+$HLA-C to C2$^+$HLA-C allotypes required a point mutation replacing asparagine with lysine at position 80. Such an MHC-C mutant could only have been subject to positive selection and increasing frequency in the population, if it were functional from its inception. In other words, it would have needed a receptor in order to survive. In the presence of cross-reactive E44 KIR that recognize both C1 and C2, such a mutant C2$^+$MHC-C would function as a ligand (98). C2$^+$MHC-C allotypes are present in gorillas, chimpanzees, bonobos, and humans (60), consistent with C2 having emerged in a common ancestor of African apes and humans. With establishment of the interaction between C2$^+$MHC-C and E44 KIR, the stage was set to evolve lineage III KIR



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

A model for evolution of the *KIR* locus in simian primates. The mouse lemur, a prosimian, has one *KIR* gene (195), suggesting that simian primate *KIR* families evolved from one ancestral *KIR* gene. The platyrrhine and catarrhine primates evolved distinctive lineages of *KIR*, as seen in the modern New World and Old World monkeys (66–68). The platyrrhine *KIR* (*yellow boxes*) form a distinctive lineage not present in other living primates. On the catarrhine lineage, five *KIR* genes emerged. These included the four framework genes: a pseudogene, a lineage I gene, a lineage II gene, and a lineage V gene (*large black boxes*). The fifth gene was of lineage III (*blue boxes*). During the evolution of the Old World monkeys, lineage II *KIR* genes expanded in the telomeric region, whereas the lineage III *KIR* gene in the centromeric region became nonfunctional (*box with X*). In the evolution of great apes, MHC-C emerged. This drove the specific expansion of lineage III *KIR* genes in the centromeric region (74, 96, 100). The specificity-determining residue at position 44 of the lineage III *KIR* is given below the gene box. K44 denotes C1-specificity, whereas M44 and T44 denote C2 specificity. In contrast, E44 KIR can be C1 specific, C2 specific, or specific for both C1 and C2. Sublineages of lineage III *KIR* (*white letter in gene box*) (**Figure 5**) present in the last common ancestor of humans and chimpanzees were differentially inherited. No C2-specific KIR was inherited by the human line. This function was recovered with the emergence of C2-specific KIR with T44. At this early stage in human evolution C1-specific KIR and C2-specific KIR were encoded in the centromeric region of different *KIR* haplotypes, which were ancestral forms of the modern *CenA* and *CenB* haplotypes. Subsequent duplication and recombination of KIR genes gave rise to ancestral and then contemporary *KIR A* and *KIR B* haplotypes. The latter can be distinguished in both the centromeric and telomeric regions. Abbreviations: KIR, killer cell immunoglobulin-like receptor; LCA, last common ancestor.

with methionine 44 (M44), a residue that confers higher specificity and avidity for C2 epitopes than was achieved by E44 KIR.

The *KIR* locus of the chimpanzee is organized similarly to that of the orangutan (**Figure 4**) but in the presence of both C1 and C2 the number of lineage III *KIR* genes encoding MHC-C receptors doubled (90, 92, 100). Diverse combinations of nine lineage III *KIR* genes and *KIR2DL5* are present in the centromeric region of chimpanzee *KIR* haplotypes (90, 100). Eight lineage III *KIR* genes encode high-avidity receptors specific for either C1 or C2 (90, 100–102). Two inhibitory and one activating C1-specific KIR have K44. Three inhibitory and one activating C2-specific KIR have M44, whereas one inhibitory C2-specific KIR has E44. That this E44 KIR does not have the cross-reactive C1+C2 specificity of the orangutan E44 KIR is due to substitutions at other positions, including cysteine at position 45 (103). The exceptional chimpanzee lineage III *KIR* gene is *KIR2DS4*, a K44 activating receptor with weak avidity for MHC class I (104). On average, an individual chimpanzee combines 1.8 inhibitory and 0.7 activating C1 receptors with 2.4 inhibitory and 0.6 activating C2 receptors. Thus, in this species C2-specific receptors have evolved to dominate the C1-specific receptors. The lineage II *KIR* gene at the telomeric end of the chimpanzee *KIR* locus encodes a receptor for epitopes of MHC-A and MHC-B that has specificity similar (90) to that of those described for rhesus macaque lineage II KIR (73).

REINVENTION OF THE *KIR* LOCUS IN ANCESTRAL HUMANS

The human and chimpanzee lineages diverged from their last common ancestor (LCA) ~8 Mya (105, 106). Anthropological evidence points to the LCA having been much more like a modern chimpanzee, or a bonobo (107), than a modern human (108–111). The implication is that following separation from the LCA, more extensive divergence from the LCA occurred on the human line than on the chimpanzee line. That is certainly true for the human *KIR* gene family, which exhibits striking and qualitative differences from both the chimpanzee and the orangutan *KIR* families.

Common to the human and chimpanzee *KIR* loci are *KIR2DS4*, *KIR2DL5*, and the framework genes. In contrast, the lineage III *KIR* genes that encode MHC-C receptors are significantly

different between the two species. In humans the variable *KIR* genes are distributed between the centromeric and telomeric regions, a feature not seen in the rhesus, orangutan, or chimpanzee *KIR* loci (74, 92, 96, 100, 112). Further distinguishing the human *KIR* locus are two functionally distinctive haplotype groups, *KIR A* and *KIR B*, defined by modules of gene and allele content in both the centromeric and the telomeric regions (112–114) (**Figure 4**). All human populations have *KIR A* and *KIR B*, but their relative frequencies vary between populations (115, 116). These haplotype differences emerged in the centromeric region soon after separation of the human and chimpanzee lines ~8 Mya (105, 112). At a later time, ~1.7 Mya, the telomeric region was populated with *KIR* genes, which came from the centromeric region, and these too evolved *KIR A* and *KIR B* characteristics (112). Many of the clinical correlations made with *KIR* track with *KIR A* and *B* differences (117, 118).

When humans and chimpanzees were first observed to have activating and inhibitory K44 C1 and M44 C2 receptors (90), it was assumed that the two species inherited the same *MHC-C* receptor genes from the LCA. However, phylogenetic analysis of the genomic regions containing each lineage III *KIR* gene did not support this model (100). The phylogenetic tree consists of five well-resolved clades (**Figure 5**): The first contains all orangutan lineage III *KIR*, the second (IIIb) contains genes encoding chimpanzee K44 C1 receptors, and the third (IIIa) contains three of the four chimpanzee genes encoding M44 C2 receptors. The fourth clade (IIIc) is characterized by shallow internal branches and contains all human lineage III *KIR*, except *KIR2DS4*. Belonging to this human-specific clade are genes encoding K44 C1 receptors, M44 C2 receptors, and T44 receptors, of which the latter are low-avidity C2 receptors. The fifth clade (IIId) includes human and chimpanzee *KIR2DS4*, one chimpanzee gene encoding an M44 C2 receptor, and the single chimpanzee gene encoding an E44 C2 receptor. The topology of this tree supports an evolutionary model in which the human MHC-C receptors were diversified on the human line and derive from only two lineage III *KIR* that were inherited from the LCA. These comprise activating *KIR2DS4* and one inhibitory lineage III *KIR*. Subsequently, the latter underwent several rounds of gene duplication and differentiation on the human line (**Figure 4**). In this model, ancestral humans inherited only one MHC-C specificity from the LCA, either C1 or C2. Because KIR recognition of MHC-C began with the C1 epitope and C1-specific KIR, and this system functioned for >10 million years before the emergence of C2, it seems more likely that a K44 C1-specific inhibitory KIR would have been preserved, along with the C1 epitope, during the population bottlenecks that accompanied the separation and speciation from the LCA. Although KIR2DS4 exhibits only weak binding to HLA class I (104) it is expressed on uterine NK cells and contributes to successful pregnancy (119), which could explain why it too was retained.

T44 is present in the human activating receptors KIR2DS3 and KIR2DS5 but has not been observed in any nonhuman primate KIR. This absence is consistent with T44 KIR having evolved specifically on the human line. That T44 confers weak C2 specificity was first shown by mutagenesis at position 44 of KIR2DL1 and KIR2DL3 (120) and then demonstrated for 6 of the 11 KIR2DS5 allotypes present in African populations (121). Of these, KIR2DS5*006 is correlated with reduced incidence of preeclampsia for pregnant women (122). Evidence for an inhibitory and educating T44 KIR comes from analysis of *KIR2DP1*, a gene paired with *KIR2DL1* in the centromeric region, which was inactivated by a nucleotide deletion, at some point during early human evolution (123). Apart from this deletion, the sequence of *KIR2DP1* appears complete and functional, and in phylogenetic trees it groups in with all the HLA-C-specific *KIR*.

Because of the strong linkage disequilibrium between *KIR2DP1* and *KIR2DL1*, inactivation of $KIR2DP1^F$ (the functional form of *KIR2DP1*) could not have proceeded by fixation of the allele in which the deletion occurred. That mechanism would have eliminated all functional polymorphism of *KIR2DL1*, an essential part of *KIR A* and *B* haplotype differences in the centromeric region.

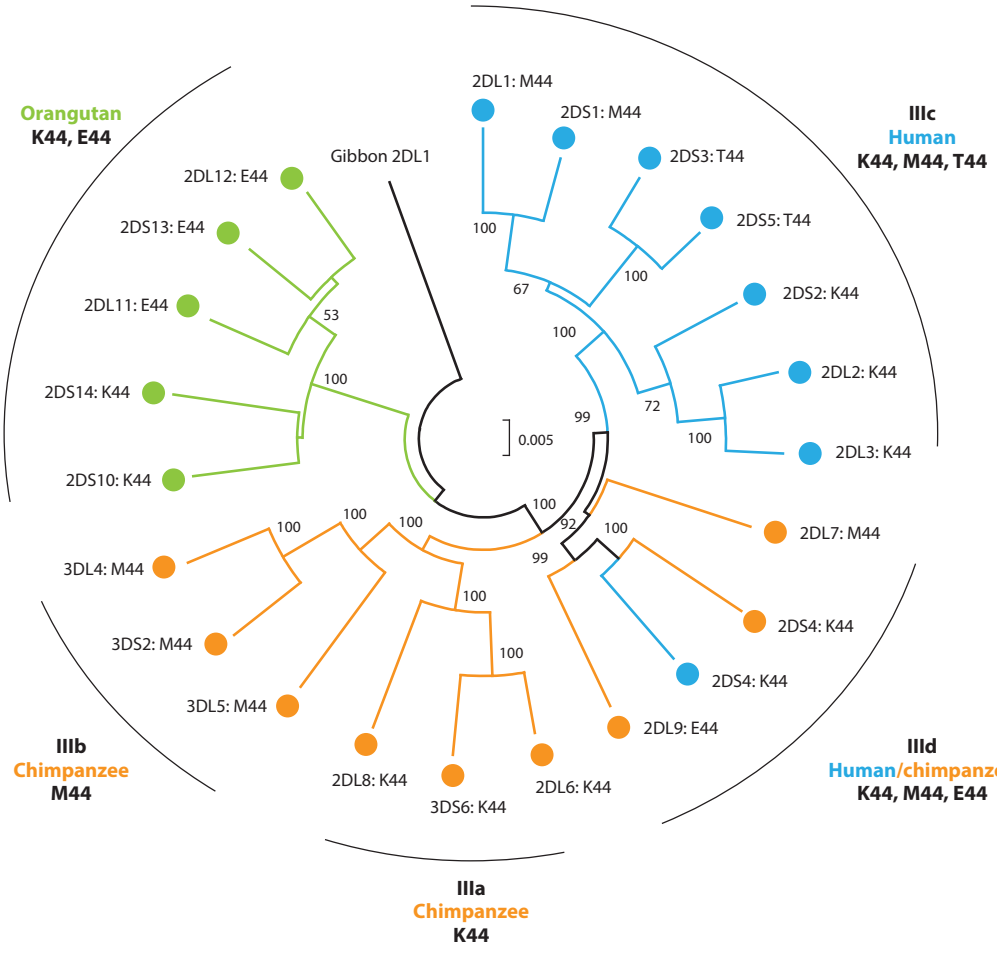


Figure 5

Phylogenetic tree of hominid lineage III *KIR*. The tree is rooted by gibbon lineage III *KIR2DL1* (196). The tree comprises five clades that are bracketed by the black curved lines. One clade is orangutan specific, one is human specific (IIIc), two are chimpanzee specific (IIIa and IIIb), and the fifth clade contains both human and chimpanzee *KIR* (IIId). The residue present at specificity-determining position 44 is indicated and marked as E44, K44, M44, or T44. (For the chimpanzee KIR3D, which have a D0 domain not present in human, orangutan, and other chimpanzee lineage III KIR, the specificity-determining position corresponds to residue 139.) Full-length genomic sequences for the *KIR* genes were aligned in Geneious (197) using the MAFFT algorithm with manual correction. Intron 1 sequences containing the microsatellite repeat element were excluded from the analysis. The neighbor-joining tree was constructed in MEGA 7 (198) (1000 bootstrap replicates, pairwise deletion, Tamura-Nei model).

Instead, small gene conversions were used to spread the deletion from one *KIR2DP1*^{F} allele to another. As a consequence of this mechanism of inactivation, the functional polymorphism of *KIR2DP1*^{F} was preserved, as well as that of *KIR2DL1* (123). The key dimorphism in KIR2DP1^{F} is at position 44, where there is an even balance of K44 and T44. Resurrected KIR2DP1^{F} allotypes show that the K44 allotypes were C1 specific and the T44 allotypes were C2 specific. Thus K44-KIR2DP1^{F} is a plausible candidate for being the one lineage III MHC-C receptor that was inherited from the LCA. Once on the human line, mutation of K44-KIR2DP1^{F} to give T44-KIR2DP1^{F} could have provided ancestral humans with their first inhibitory C2-specific

receptor (**Figure 4**). In a manner not seen in the modern HLA-C receptors, the *KIR2DP1*^{F} gene encoded both C1-specific and C2-specific inhibitory receptors. This system of stronger K44 C1 receptors and weaker T44 C2 receptors could have provided a stopgap intermediate, until a stronger M44 C2 receptor emerged. Alternatively, weak T44 C2 receptors could have been advantageous at this early stage of human evolution when all variable *KIR* genes were still located in the centromeric region (123).

HLA HAPLOTYPES FAVOR THE EDUCATION OF EITHER C94:NKG2A^{+} NK CELLS OR KIR^{+} NK CELLS

Human NK cells are educated by two complementary systems of HLA class I ligands and inhibitory NK cell receptors. These comprise the older, conserved interaction of HLA-E with CD94:NKG2A and the younger, extraordinarily diverse interactions of KIR with subsets of HLA-A, -B, and -C allotypes. KIR-mediated education of NK cells emerged in the simian primates and from the beginning has had its influence on the evolution of CD94:NKG2A^{+}-mediated education.

A defining feature of MHC-E is the specificity of its peptide-binding site (124). Physiologically, the only peptides that load this site are nonamers derived from the leader sequences of MHC class I that have a methionine anchor at position 2. These nonamers correspond to residues −22 to −14 of the MHC class I polypeptide. On binding such peptides in the endoplasmic reticulum, HLA-E becomes properly folded and moves to the cell surface, where it can be recognized by the CD94:NKG2A receptor on NK cells (38). In Old World monkeys and orangutans all MHC class I leader peptides have methionine at position −21 (−21M) (43). For these species the amount of MHC-E at the surface of a cell provides a measure of the total amount of MHC-A and -B (Old World monkeys) or MHC-A, -B, and -C (orangutans) being made in the cell. Educated CD94:NKG2A^{+} NK cells use this measure to distinguish unhealthy cells, displaying abnormally low levels of MHC class I, from healthy cells exhibiting a normal level of MHC class I. In humans, −21M is fixed at HLA-A and HLA-C, but at position −21 of HLA-B most allotypes have threonine (−21T) and only a minority have methionine (−21M) (43). Nonamer peptides cleaved from the leader sequences of −21T HLA-B are not effective in binding HLA-E and bringing it to the cell surface to engage CD94:NKG2A. Because HLA-B is expressed more highly than HLA-A or HLA-C, the −21M/T dimorphism has considerable influence on cell surface expression of HLA-E.

Individuals homozygous for −21M (M/M) have higher surface expression of HLA-E than individuals homozygous for −21T (T/T) (43). Heterozygotes (M/T) have intermediate expression, but it is much closer to M/M than T/T, a dominance of −21M observed for other functional properties. This hierarchy is consistent with the supply of HLA-E-binding peptides being highest for M/M and lowest for T/T individuals. Dimorphism at position 107 of HLA-E also affects its surface expression (125), with glycine giving higher expression than arginine, but this difference is of lesser magnitude than that caused by the position −21 HLA-B dimorphism (43). Higher expression of HLA-E increases the education of CD94:NKG2A^{+} NK cells in M/M individuals relative to T/T individuals. This is reflected in the number of educated cells, the diversity of their surface phenotype, and their increased capacity to mediate the missing-self response, antibody-dependent cellular cytotoxicity (ADCC) and secretion of inflammatory cytokines (43). The −21 *HLA-B* dimorphism divides the human population into three groups comprising 6% M/M, 32% M/T, and 62% T/T. Because of the dominance of −21M, the three genotypes can for simplicity be considered to represent two functional groups, defined by the presence or absence of −21M. Thus 38% of humans have −21M (M/M+M/T) and well-educated CD94:NKG2A^{+} NK cells, whereas 62% lack −21M (T/T) and have CD94:NKG2^{+} NK cells that are less educated (43). This

functional dimorphism is analogous to that observed for *KIR A* and *KIR B* haplotypes, in which the main functional differences are defined by the presence or absence of *KIR B* (126, 127). Clinical correlation with −21 HLA-B dimorphism has been made in the study of partners comprising one who is HIV infected and one who is not, despite chronic exposure to the virus. Such resistance to HIV infection correlates genetically with the *−21T/T* genotype and functionally with more effective killing of HIV-infected cells by CD94:NKG2A$^+$ NK cells (128, 129).

The class I region of the human *MHC* consists of duplicated blocks of ∼100 kb, each containing an *HLA class I* gene (130). *HLA-B* and *HLA-C* are adjacent genes in the *HLA class I* region and separated by 82 kb (131). They were likely formed by duplication of an *HLA-B*-like ancestor. The *−21M/T* polymorphism in *HLA-B* is in strong linkage disequilibrium with other functional polymorphisms of *HLA-B* and *-C*. For example, HLA-B allotypes that carry the Bw4 epitope almost never have −21M (43). The one exception is *HLA-B*38:01*, which is considered to have entered the modern human population by introgression from an archaic human population (132) (**Figure 1**). Overall, the consequence of this linkage disequilibrium is that Bw4$^+$ HLA-B, which is dedicated to educating KIR3DL1$^+$ NK cells, does not contribute to HLA-E-mediated education of CD94:NKG2A$^+$ NK cells. In Eurasians, HLA-C allotypes that carry the C2 epitope almost never have HLA-B with −21M (43). Consequently, the haplotypes encoding C2$^+$HLA-C, the educating ligand for KIR2DL1$^+$ NK cells, have HLA-B that does not contribute to the education of CD94:NKG2A$^+$ NK cells. Such linkage disequilibrium does not occur in sub-Saharan African populations, in which three common African-specific haplotypes combine *C2$^+$HLA-C* with *−21M HLA-B* (43). All three *HLA-B* and two of the three *HLA-C* alleles on these haplotypes are specific to African populations and have yet to be studied in any depth. One possible explanation for this striking difference between Eurasians and Africans is that haplotypes combining *C2$^+$HLA-C* with *−21M HLA-B* were not present in the human populations that migrated out of Africa. Alternatively, these haplotypes may have been taken out of Africa by human migrants but subsequently have been lost, either through genetic drift or through negative selection.

HLA-C expression is affected by a micro-RNA (miR-148a) that binds to a site in the 3′ untranslated region of *HLA-C* and reduces gene expression (133). Polymorphism at this site arises from a nucleotide deletion that prevents miRNA binding, thereby increasing HLA-C expression. This polymorphism in *HLA-C* is in complete linkage disequilibrium with the *HLA-B* polymorphism at position −21 (43). As a consequence, the C1$^+$HLA-C allotypes encoded by *−21M* haplotypes are generally expressed at lower levels than the C2$^+$HLA-C encoded by *−21T* haplotypes. More effective C1-mediated education of KIR2DL2/3$^+$NK cells is therefore provided by haplotypes that do not contribute to HLA-E-mediated education of CD94$^+$ NK cells. From this we see that the genomic region containing *HLA-B* and *HLA-C* maintains a critical balance between two haplotype groups: one biased to providing ligands that educate CD94:NKG2A$^+$ NK cells, and the other biased to providing ligands that educate KIR$^+$ NK cells. This dimorphism of *HLA B-C* haplotypes is likely to have many clinical correlations (128, 129). As was first appreciated by Yunis et al. (134), the challenge will be in distinguishing the contributions of the various and functionally distinct factors that are in such strong linkage disequilibrium.

In this aspect of NK cell immunogenetics, the chimpanzee provides an intriguing contrast to the human condition. In chimpanzee *MHC* haplotypes *−21M* is fixed at *MHC-A*, *−21T* is fixed at *MHC-B*, and *MHC-C* exhibits *M/T* polymorphism (43). Thus chimpanzee MHC-E-mediated education of CD94:NKG2A$^+$ NK cells has been more extensively replaced by MHC-B- and MHC-C-mediated education of KIR$^+$ NK cells, than in humans. This correlates with the greater diversity and stronger functions of chimpanzee KIR, compared to their human counterparts (101, 102).

REPRODUCTION DRIVES THE CO-EVOLUTION OF *MHC-C* AND *KIR*

KIR A and *B* haplotypes were first defined on the basis of *KIR* gene content (113, 114). *KIR A* has conserved gene content in which the centromeric region contains *KIR2DL3*, *2DP1*, and *2DL1* and the telomeric region contains *KIR3DL1* and *2DS4*, the only *A* haplotype gene encoding an activating receptor. A shorter variant of the *A* haplotype lacks the paired *KIR2DP1* and *2DL1* genes (135) (**Figure 6**). Gene content diversity characterizes the *KIR B* haplotypes. Defining a *B* haplotype is one or more of the *KIR2DL1*, *2DL5*, *2DS1*, *2DS2*, *2DS3*, and *2DS5* genes. Of these *B* haplotype genes, *KIR2DL2* and *2DS2* are in the centromeric region, *KIR3DS1* and *2DS1* are in the telomeric region, and *KIR2DL5*, *2DS3*, and *2DS5* can be in either of the two regions or in both of them (75, 112, 135, 136).

Between the *KIR3DP1* and *2DL4* framework genes is a repetitive sequence, a hotspot for recombination that has created hybrid haplotypes, either combining the centromeric region of an *A* haplotype (*CenA*) with the telomeric region of a *B* haplotype (*TelB*) or the centromeric region of a *B* haplotype (*CenB*) with the telomeric region of an *A* haplotype (*TelA*) (112, 135, 136). The short, homologous sequences separating *KIR* genes are sites where asymmetric recombination has produced numerous variant haplotypes by gene duplication, deletion, or fusion (135, 136). Most of these haplotypes are rare. The smallest *KIR* haplotype has 4 genes, and the largest has 20 genes (135, 136).

Allelic variation also distinguishes *KIR A* and *B* haplotypes. Thus *CenA* encodes strong KIR2DL1 allotypes, whereas *CenB* encodes attenuated KIR2DL1 allotypes with weak affinity or impairment of surface expression or signaling function (120). Conversely, the KIR2DL2 allotypes encoded by *CenB* tend to be stronger C1 receptors than the KIR2DL3 allotypes encoded by *CenA*. A similar trend is seen in the segregation of *KIR2DP1* alleles. Only high-affinity K44 KIR2DP1^{F} allotypes were encoded on *CenA*, whereas *CenB* encoded all of the low-affinity T44 KIR2DP^{F} allotypes, of which half have no inhibitory signaling function, because of premature termination in the cytoplasmic tail (123). Differences between *TelA* and *TelB* can be similarly dramatic. The *KIR3DL1/S1* gene encodes two lineages of qualitatively different allotypes. *TelA* encodes Bw4-specific KIR3DL1 allotypes that are polymorphic, inhibitory, educating receptors, whereas *TelB* encodes KIR3DS1, a conserved activating receptor that does not recognize Bw4 but interacts with open conformers of conserved HLA-F (137, 138).

Although *KIR* and *HLA-A*, *-B*, and *-C* genes are highly polymorphic they vary in different ways (**Table 1**). The variable α_1 and α_2 domains of HLA-A, -B, and -C allotypes differ on average by 17, 18, and 14 amino acid substitutions, respectively. Thus recombination has inserted a pool of substitutions into many different and divergent combinations (139). In contrast, the variable D1 and D2 domains of the lineage III KIR differ by 2–5 substitutions. Similarly the D0, D1, and D2 domains of the lineage II KIR differ by 2–10 substitutions (**Table 1**). One likely cause of this difference is the relative age of the various genes. With the exception of *KIR2DS4*, all of the lineage II and III *KIR* genes are human specific and thus <8 million years old. In contrast, *MHC-A* and *-B* are at least 35–49 million years old, and *MHC-C* is 21–28 million years old (89, 140).

Emerging from the earliest correlations made of *KIR* with disease were pointers that human-specific evolution of the *KIR A* and *B* haplotype difference was a consequence of different requirements needed for NK cells to function in immune defense and reproduction (141). Thus homozygosity for *KIR A* and *C1*$^{+}$*HLA-C* correlated with better prognosis for hepatitis C virus infection (142–144), whereas the same compound genotype increased the risk of preeclampsia, for pregnant women who carry a *C2*$^{+}$*HLA-C* fetus (145). Distinctive populations of NK cells function in viral defense and reproduction. During viral infection, CD16^{+}CD56^{dim} NK cells from the peripheral blood enter infected tissue and respond by killing infected cells and secreting

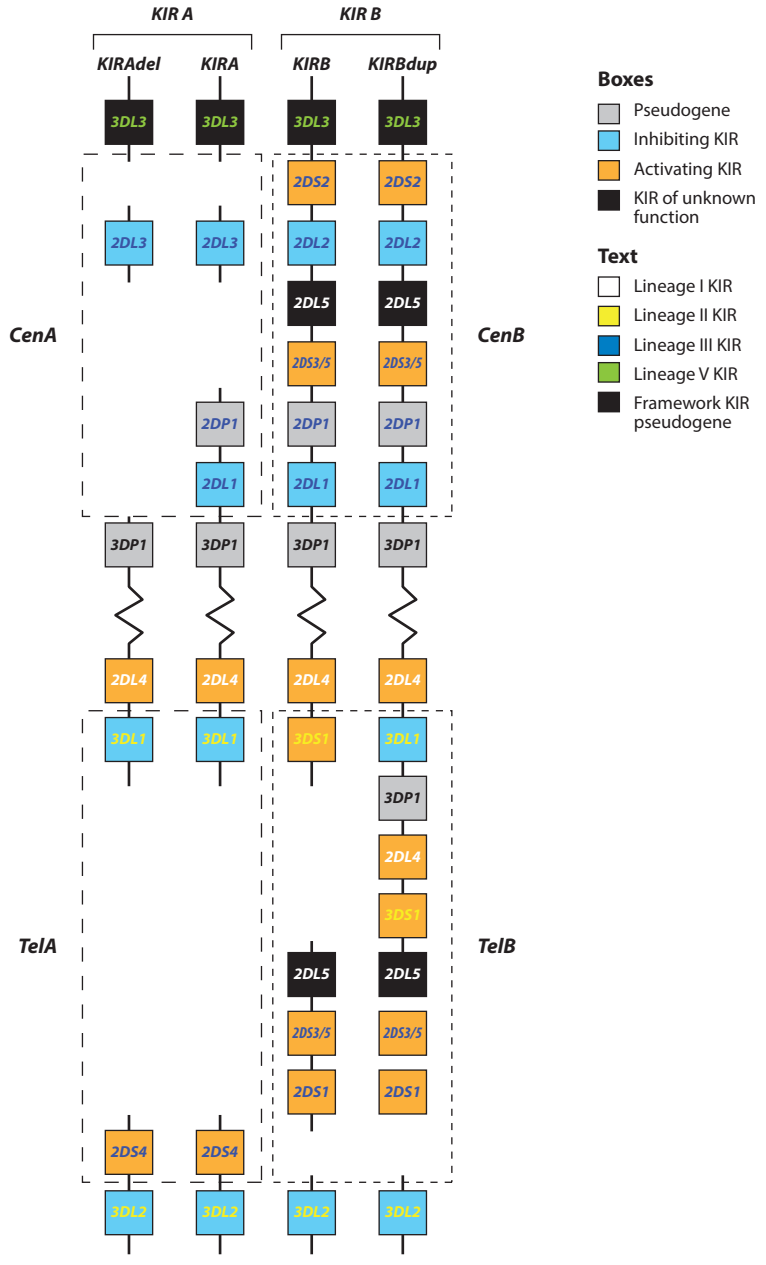


Figure 6

KIR A and *KIR B* haplotypes of the human *KIR* locus. *KIRA* is the common, canonical *A* haplotype. *KIRAdel* is the shorter variant *A* haplotype, which was formed by deletion of the paired *KIR2DP1* and *KIR2DL1* genes (30). *KIRB* is a common form of *B* haplotype. *KIRBdup* is a longer variant formed by a duplication that introduced *KIR3DS1* and additional copies of *KIR2DL4* and *KIR3DP1* into the telomeric region (199). *CenA* and *CenB* are centromeric regions of the *A* and *B* haplotypes, respectively. *TelA* and *TelB* are the telomeric regions of the *A* and *B* haplotypes, respectively. The zigzag between the centromeric and telomeric regions denotes a hotspot for reciprocal recombination.

Table 1 Distinctive *HLA* class I and *KIR* polymorphisms in human populations[a]

		Number of alleles in IPD	Number of coding sequence alleles	Number of coding sequence allotypes	Average pairwise difference		Length of sequence	
					Nucleotide	Amino acid	Allele (bp)	Allotype (aa)
KIR2DL1		59	43	34	6.2	4.5	1,047	348
KIR2DS1		16	11	9	2.9	2.1	915	304
KIR2DL2		31	19	14	5.4	4.3	1,047	348
KIR2DL3		59	46	35	5.3	4.0	1,026	341
KIR2DS2		23	12	10	5.3	3.4	915	304
KIR2DS3		16	13	8	2.2	1.7	915	304
KIR2DS4		35	23	10	3.1	3.4	915	304
	FL		11	10	2.1	3.4	915	304
	del		12	NA	4.0	NA	893	NA
KIR2DS5		23	19	17	3.5	2.7	915	304
KIR2DP1		40	22	NA	6.2	NA	1,049	NA
KIR3DP1		29	21	NA	2.6	NA	949	NA
	FL		12	NA	2.6	NA	949	NA
	del		9	NA	2.5	NA	913	NA
KIR3DL1		137	99	79	9.3	7.2	1,335	444
	w/3DL1/2		106	86	12.2	9.9	1,368	455
KIR3DS1		39	29	21	2.5	2.3	1,165	383
KIR3DL2		158	148	111	4.4	3.9	1,368	455
KIR2DL4		65	56	31	5.2	4.7	1,134	377
KIR2DL5		51	37	22	4.8	3.2	1,128	375
KIR3DL3		126	112	74	6.2	4.1	1,233	410
HLA-A	ALL	3,967	3,684	2,934	24.3	17.7	1,098	365
	E2E3		3,391	2,579	23.0	17.2	546	181
	FL		510	409	36.8	24.2	1,098	365
HLA-B	ALL	4,827	4,538	3,758	28.2	18.0	1,092	363
	E2E3		4,259	3,320	27.7	17.8	546	181
	FL		645	526	35.5	21.7	1,092	363
HLA-C	ALL	3,577	3,328	2,639	17.8	12.2	1,119	369
	E2E3		3,080	2,307	16.7	11.6	546	181
	FL		643	528	29.9	20.1	1,119	369
HLA-E	ALL	26	13	8	2.0	1.7	1,077	358
	E2E3		7	4	2.0	1.7	546	181
	FL		7	5	2.0	1.8	1,077	358
HLA-F	ALL	25	6	5	1.7	1.6	1,041	346
	E2E3		2	2	1.0	1.0	546	181
	FL		6	5	1.7	1.6	1,041	346
HLA-G	ALL	56	43	18	3.6	2.4	1,017	338
	E2E3		34	15	3.0	2.3	546	181
	FL		18	6	4.4	1.9	1,017	338

[a]Shown are the mean pairwise differences in sequence for alleles and allotypes of the *KIR* and HLA class I genes. *KIR2DS4* has two divergent lineages of alleles, one that is full length (FL) and one that has a deletion (del) causing premature termination of translation (200). *3DL1/2* is a fusion gene formed by recombination between *KIR3DL1* and *KIR3DL2* (201). For the *HLA* sequences only coding sequence was compared. ALL denotes comparison of all alleles regardless of sequence length, E2E3 denotes comparison encompassing only the sequences of exons 2 and 3, which encode the variable α_1 and α_1 domains. FL denotes comparisons within a reduced dataset including only alleles for which the complete coding sequence is known. In all datasets, duplicate sequences (alleles having variation outside the scope of the analysis, but otherwise identical to another allele) were removed.

inflammatory cytokines. At the very beginning of pregnancy, $CD16^{-}CD56^{dim}$ NK cells become the dominant leukocytes in the uterine tissue; there they interact with the fetal extravillous trophoblast cells (146, 147). Uterine NK cells do not kill the trophoblast cells but act as helper cells that enable trophoblast cells to participate in placentation.

Preeclampsia is a hypertensive disorder caused by poor placentation (148). During embryo implantation a mother's uterine NK cells interact with fetal extravillous trophoblast cells, causing them to invade the uterine tissue and modify the spiral arteries by replacing smooth muscle and endothelial cells with trophoblast. Such remodeling converts the narrow arteries into large conductance vessels suitable for supplying the placenta with blood and nourishment until term. Controlling this process are the interactions of maternal NK cell receptors with the maternal and paternal HLA-C, E, F, and G ligands expressed on the trophoblast (149). HLA-G is expressed only on extravillous trophoblast cells and is a high-affinity ligand for LILRB2 (150, 151), a leukocyte immunoglobulin-like receptor, as well as a low-affinity ligand for KIR2DL4. Whereas other KIR bind HLA class I ligands at the NK cell surface, KIR2DL4 activates NK cells by engaging soluble forms of HLA-G in NK cell endosomes (152). Interaction between isolated HLA-G and KIR2DL4 has yet to be detected in direct binding assays (153–155). Functional interaction of KIR2DL4 with HLA-G induces a state of senescence in NK cells, during which they secrete the proinflammatory cytokines, proangiogenic factors, and other mediators that break down the extracellular matrix, thereby facilitating trophoblast invasion and remodeling of the spiral arteries (156). Because HLA-A and -B are not expressed by extravillous trophoblast, HLA-C is the one highly polymorphic ligand that contributes to these interactions and thus to variation in human pregnancy. Poor placentation, the result of insufficient invasion of the uterus, can cause stillbirth, miscarriage, preterm labor, fetal growth restriction, preeclampsia, and eclampsia. At the other end of the spectrum of pregnancy disorders, excessive invasion results in overly large babies that can cause obstructed labor and other complications associated with giving birth (148).

Poor placentation is probably the consequence of insufficient cooperation by uterine NK cells and extravillous trophoblast cells. The disorders caused by poor placentation are mainly ones of a first pregnancy, providing subsequent pregnancies involve the same father. This phenomenon argues for the maternal immune system being able to adapt to allogeneic HLA-C of the father. In pregnant women who are homozygous for *KIR A* and $C1^{+}$HLA-C the uterine NK cells are educated by $C1^{+}$HLA-C, but not by $C2^{+}$HLA-C. Upon implantation of an embryo that expresses $C1^{+}$HLA-C of maternal origin and $C2^{+}$HLA-C of paternal origin, the maternal NK cells will confront trophoblast cells expressing both self $C1^{+}$HLA-C and allogeneic $C2^{+}$HLA-C. The mother's NK cells, having been educated by homozygous $C1^{+}$HLA-C cells, have the potential to make a missing-self response to the reduced expression of $C1^{+}$HLA-C on heterozygous trophoblasts. Only a fraction, ~20%, of the peptides bound by $C1^{+}$HLA-C permit recognition by KIR2DL3, the C1-specific receptor of the *KIR A* haplotype (157, 158). So differences between the peptides bound by $C1^{+}$HLA-C on fetal trophoblast and on the maternal $C1^{+}$HLA-C cells that educated the NK cells also have the potential to provoke a missing-self response. Another possible contributory mechanism is the interaction between allogeneic $C2^{+}$HLA-C on the trophoblast and C2-specific KIR2DL1 on uterine NK cells that inhibits the mutual activation of NK cells and trophoblast cells. Here the inhibition mediated by the noneducating KIR2DL1 could reduce or override the missing-self response mediated by the educating receptor, KIR2DL3. Within this overall context, maternal NK cells are likely to be educated by paternal $C2^{+}$HLA-C during the course of a first pregnancy and in this way reduce the risk of deficient placentation in subsequent pregnancies (159).

A *TelB* haplotype that is common in Europeans, and rare in Africans, contains the *KIR3DS1*, *2DL5*, *2DS5*, and *2DS1* genes (115, 160). *KIR2DS1* encodes an activating C2-specific receptor with

avidity for C2 that is half that of KIR2DL1*003, the most frequent KIR2DL1 allotype (120). Pregnant women who have both C2$^+$HLA-C and KIR2DS1 experience a lower incidence of preeclampsia than women who have only C2$^+$HLA-C or KIR2DS1. It is unlikely that KIR2DS5*002 encoded by this haplotype contributes to the protection, because this allotype cannot recognize C2 (121). Whereas European populations effectively have 1 KIR2DS5 allotype, African populations have a rich and balanced polymorphism of 11 allotypes; of these, 7 recognize C2 and 4 do not (121). In a pioneering clinical study, the combination of C2$^+$HLA-C and KIR2DS5*006, a C2-binding allotype, was seen to reduce the incidence of preeclampsia (122). And as a group, the C2-binding KIR2DS5 allotypes were also protective (121). The striking differences between the activating C1 receptors of Europeans and Africans support a model in which modern humans-out-of-Africa lost functional KIR2DS5 and gained functional KIR2DS1 by introgression of the *KIR3DS1–2DL5–2DS5–2DS1 TelB* region from archaic humans (132).

During implantation of rhesus macaque embryos, uterine invasion is limited to the endothelium of the spiral arteries. Thus a more extensive invasion of tissue and blood vessels is characteristic of humans and great apes, the species that have *MHC-C*. Humans (161), chimpanzees (162, 163), and gorillas (164) exhibit the most extensive invasion (165), which is influenced by the *KIR* genotype of the mother and the *MHC-C* genotypes of both parents. Uterine invasion has yet to be studied in the orangutan (165). However, given that *MHC-C* is limited to the *C1* epitope and to 50% of *MHC* haplotypes, the prediction is that uterine invasion in orangutan pregnancy is generally less than in human or chimpanzee pregnancy.

EPISODES OF SELECTION FOR NOVEL *KIR* IN HUMAN POPULATIONS

Modern humans originated in Africa around 200,000 years ago, where they extended their range for >100,000 years, before starting to migrate out of Africa ~100,00 years ago (166–168). Eventually, modern humans populated all other continents with the exception of Antarctica. The genetic diversity in contemporary human populations varies inversely with the distance from their homeland to East Africa, the site favored for human origins. Thus sub-Saharan Africans have the greatest diversity and South Amerindians the least, ~60–70% of that in Africans (169–172). This trend is reflected in the population diversity of the *KIR* and *HLA class I* genes, as assessed by the number of alleles observed for the polymorphic genes (**Table 2**). For human populations worldwide there is an inverse correlation between the frequencies of the *KIR A* haplotype and *C2$^+$HLA-C* (145, 173, 174). Because *KIR A/A* mothers carrying a *C2$^+$HLA-C* fetus are at risk of poor placentation, this correlation is consistent with preeclampsia, and the other disorders arising from poor placentation, having been a major selective force on the variation of *KIR* and *HLA-C*. Such effects have been observed in the KhoeSan hunter-gatherers of southern Africa and the Yucpa, indigenous to South America, populations that represent the beginning and end of one of the longest pathways of ancient human migration (175).

KhoeSan: An African Population in Which C2$^+$HLA-C Is at High Frequency

Characteristic of the KhoeSan and other African populations is a high frequency of *C2$^+$HLA-C*, which can exceed that of *C1$^+$HLA-C* (**Table 2**). With a frequency of 63%, *C2$^+$HLA-C* is particularly high in the KhoeSan (176) and thus has the potential to give rise to an increased incidence of poor placentation. The KhoeSan have ten alleles of *KIR2DL1*, the inhibitory receptor implicated in poor placentation, and they have relatively even frequencies. Of these, *KIR2DL1*022* and

Table 2 Comparison of HLA epitopes and their cognate KIR in five human populations[a]

		Africa		Europe	Asia	South America
		KhoeSan	Ghanaian	Northern Irish	Japanese	Yucpa
C1⁺ HLA-C allotypes						
	Number	12	12	14	4	3
	Frequency (%)	37	44	67	92	83
C2⁺ HLA-C allotypes						
	Number	8	11	7	4	2
	Frequency (%)	63	56	33	8	17
KIR2DL1 alleles						
	Number	10	10	6	3	1
KIR2DL2/3 alleles						
	Number	9	9	6	4	5
Strong KIR2DL1 (%)		28	49	68	96	46
Weak KIR2DL1 (%)		32	28	19	1	0
Inactive (%)		21	0	0	0	0
Blank (%)		18	23	14	3	56

[a]Shown are comparisons of the numbers of *KIR2DL1* and *KIR2DL2/3* alleles and their ligands (C2⁺HLA-C and C1⁺HLA-C, respectively) from five worldwide populations for which allele-level typing for *KIR* was available (120). At the bottom of the table the frequencies of functional categories of KIR2DL1 are shown.

*KIR2DL1*026* evolved in the KhoeSan during the last 100,000 years and have unique properties suggesting they were selected for improving placentation and reproductive success (177).

In the KhoeSan *KIR2DL1*026* arose by point mutation of *KIR2DL1*001*, and *KIR2DL1*022* arose by point mutation of *KIR2DL1*012*. Both KIR2DL1*001 and KIR2DL1*012 are high-affinity, inhibitory C2-specific receptors. In contrast, *KIR2DL1*026* has a stop codon at the end of the transmembrane region that eliminates all inhibitory signaling function while maintaining some cell surface expression. Thus KIR2DL1*026 cannot function in NK cell education, but it recognizes C2⁺HLA-C and might have some role as an adhesion molecule (177).

The KIR2DL1*022 allotype has a point substitution in codon 44 that replaces methionine 44 with lysine 44. The functional effect of this change, located in the KIR binding site for HLA-C, is to make 2DL1*022 an inhibitory C1 receptor. Moreover, KIR2DL1*022 has both a higher avidity and a higher specificity for C1 than any KIR2DL2 or KIR2DL3 allotype. As a consequence of these qualitative and quantitative differences, *KIR* haplotypes that carry *KIR2DL1*022* do not encode an inhibitory C2-specific KIR but have two genes encoding inhibitory C1-specific KIR (177).

*KIR2DL1*026* and *KIR2DL1*022* have both risen to higher frequencies in the KhoeSan population than their parental alleles. This is consistent with these new variants having been subject to positive natural selection. Together the two new variants comprise 21.4% of the *KIR2DL1* alleles. The *CenB* haplotypes that contain *KIR2DL1*026* or *KIR2DL1*022* exhibit strong linkage disequilibrium and are highly conserved, whereas that is not so for all haplotypes carrying other *KIR2DL1* alleles. This difference reflects both the recent origin of *KIR2DL1*026* and *KIR2DL1*022* and the speed at which selection increased their frequencies in the KhoeSan population (177).

As only 14% of the KhoeSan lack C2$^+$HLA-C, ~86% of pregnancies are estimated to involve mothers whose uterine NK cells have been previously exposed to self C2$^+$HLA-C. Segregating in the KhoeSan are three *KIR* haplotypes that do not encode an inhibitory C2-specific receptor. These comprise the *KIR B* haplotypes that carry either *KIR2DL1*022* or *KIR2DL1*026* and those that lack the *KIR2DL1* and *KIR2DP1* genes. Together these haplotypes represent ~40% of all KhoeSan *KIR* haplotypes (177). Assuming Hardy-Weinberg equilibrium, ~15% of KhoeSan pregnancies are predicted to involve mothers who lack an inhibitory C2-specific KIR, and ~48% to involve mothers having only one copy of *KIR2DL1*. The former are predicted to be resistant to poor placentation caused by paternal C2$^+$HLA-C, and the latter to have some measure of protection.

Yucpa: A South Amerindian Population in Which C1$^+$HLA-C Is at High Frequency

The Yucpa is a small indigenous population from the Sierra de Perijá, a mountain range at the border between Venezuela and Colombia. The Yucpa have six *HLA-A*, seven *HLA-B*, and six *HLA-C* alleles (178). Two of the HLA-A and five of the HLA-B allotypes are specific to Amerindians, whereas the three C1$^+$HLA-C and two C2$^+$HLA-C allotypes are all of Eurasian origin. This difference likely reflects, and is evidence for, the qualitative difference in the physiological functions of HLA-C in reproduction and of HLA-A and -B in immunity. The role of HLA-A and -B is to provide innate and adaptive immunity against infection with viruses and other intracellular pathogens. The rapid evolution of many such pathogens selects for new HLA-A and -B variants that can provide better immunity than the old variants to which the pathogen has adapted (179–181). Maintenance of effective immunity is needed at all times in all members of a human population. In contrast, the variables that affect and influence the role of HLA-C in placentation are ones of human anatomy and physiology (182), which are more slowly evolving than microbial pathogens. Under such selective pressures HLA-C evolves more slowly than HLA-A and -B.

The genomes of South Amerindians have around 60–70% (169–172) of the unselected, neutral diversity of African genomes. This difference reflects the population bottlenecks that occurred during human migration from Africa to South America via Eurasia. *HLA-C*07:02* is a common allele in Eurasian populations, uncommon in Africans, and present in a Neanderthal genome, suggesting modern Eurasian populations acquired this allele through introgression from a Neanderthal (132). That modern and Neanderthal humans were capable of successful reproduction, despite their ~500,000 years of separation (183), is also evidence for slower change in the selection pressures on reproduction than immunity. Subsequently, migrants from Eastern Asia carried C1$^+$C*07:02 to South America, where it reached a frequency of 76% in the Yucpa, the highest worldwide (177, 178). Three other C1$^+$HLA-C allotypes (C*03:02, C*03:04, and C*01:02) are present in the Yucpa that, with C*07:02, give an allele frequency of 82% for C1$^+$HLA-C (178).

The abundance of C*07:02 and the C1 epitope in the Yucpa has induced changes in KIR2DL3, the inhibitory C1 receptor encoded on *KIR A* haplotypes. Of the three KIR2DL3 allotypes present in the Yucpa, KIR2DL3*001 was brought from Eurasia, whereas KIR2DL3*009 and KIR2DL3*008N are Yucpa specific and evolved from KIR2DL3*001 by different mutations (178). A deletion in 2DL3*008N prevents a functional protein from being made, whereas a substitution of proline for arginine at position 148 in 2DL3*009 reduces its avidity for C*07:02 to ~65% that of 2DL3*001.

The Yucpa have an even balance of 46% *KIR A* and 54% *KIR B* haplotypes. Among the *KIR A* haplotypes 16% lack a functional *KIR2DL3* gene, 75% encode KIR2DL3 with reduced avidity for C1$^+$HLA-C, and 9% have retained high affinity KIR2DL3*001. Thus the educating inhibitory C1

receptor has been eliminated or attenuated on 91% of the *KIR A* haplotypes. In contrast the Yucpa *KIR B* haplotypes all encode high-avidity inhibitory C1 receptors, which are either KIR2DL2*003 (92.5% of haplotypes) or KIR2DL3*001 (7.5% of haplotypes). Thus the attenuation of inhibitory C1 receptors has been targeted specifically to the *KIR A* haplotypes. Furthermore, there has been no attenuation of C2-specific KIR2DL1 on *KIR A* haplotypes, which all encode KIR2DL1*003, the high-avidity C2 receptor that is common in Eurasians. In dramatic contrast to the *KIR A* haplotypes, 92% of the Yucpa *KIR B* haplotypes lack the paired *KIR2DL1* and *KIR2DP1* genes. The other 8% of KIR haplotypes carry *KIR2DL1*003*. The extreme attenuation of *KIR2DL1* on Yucpa *KIR B* haplotypes was not achieved by selection for alleles encoding weaker forms of KIR2DL1, but by driving one *KIR* haplotype that lacks the *KIR2DL1* gene to high frequency (88%) (178).

In the Yucpa the frequency of women having the *KIR A/A* genotype is 21% and the frequency of men having $C2^+$HLA-C is 33% (178). In a model where HLA and KIR have no effect on mate choice (184–187), only 7% of pregnancies would combine a $C2^+$HLA-C father with an *A/A* mother, the combination associated with poor placentation. If HLA and/or KIR do influence mate choice the frequency of pregnancies with a $C2^+$ father and an *A/A* mother might be further reduced. Further reducing the likelihood of poor placentation, the dominant *B* haplotype combines absence of *KIR2DL1* with presence of *KIR2DS1*, the activating C2 receptor. The combination of maternal KIR2DS1 and fetal $C2^+$HLA-C protects against poor placentation and is associated with higher birth weight (148). In this context of a small isolated population with a history of bottlenecks, there were likely to have been strong pressures on reproduction. Such pressures have largely replaced $C2^+$HLA-C with $C1^+$HLA-C and caused the domination of a *KIR B* haplotype that has *KIR2DS1* but lacks *KIR2DL1*. In this context, attenuation of the KIR2DL3 encoded by *KIR A* haplotypes could have been a response to the increased surface expression of $C1^+$HLA-C on cells of individuals homozygous for $C1^+$HLA-C, the majority of individuals in the Yucpa population.

Of four Yucpa $C1^+$HLA-C allotypes, only HLA-C*07:02 was driven to high frequency, suggesting some specific property of HLA-C*07:02 contributed to this selection. A candidate is the presence of alanine at position −21, which prevents the production of peptide that can bind HLA-E and engage CD94:NKG2A (188, 189). Thus the high frequency of HLA-C*07:02 means that HLA-C does not contribute to the education of CD94:$NKG2A^+$ NK cells in a majority of Yucpa individuals. Balancing this effect is an unusually high frequency, 76%, of *−21M HLA-B* alleles in the Yucpa, with only 6% of individuals lacking *−21M HLA-B*. This statistic implies that education of CD94:$NKG2A^+$ NK cells is particularly strong in the Yucpa population.

SUMMARY

Comparison of KIR and MHC class I in humans and nonhuman primates shows this interacting system of ligands and NK cell receptors is specific to the simian primates. The difference between species points to the rapid evolution of these systems, subject to strong competing pressures from immunity and reproduction. The unprecedented genetic polymorphisms of *HLA class I* and *KIR* are testament to the numerous compromises made during the evolution of human species and their constituent populations. During early human history a significant part of the system was lost and then evolved anew. Study of indigenous and anthropologically well-characterized human populations has proved uniquely informative in showing the individual steps by which the ligands and receptors coevolve. Each population studied has contributed something different to the big picture, emphasizing the value of future high-resolution analysis of *HLA* and *KIR* in human populations for which demographic history is known.

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