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How did variable NK-cell receptors and MHC class I ligands influence immunity, reproduction and human evolution?

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Preface

Natural killer (NK) cells have roles in immunity and reproduction that are controlled by variable receptors that recognize MHC class I molecules. The variable NK cell receptors found in humans are specific to simian primates, where they have progressively co-evolved with MHC class I molecules. The emergence of MHC-C in hominids drove the evolution of a system of MHC-C receptors that is most elaborate in chimpanzees. In contrast, the human system appears to have been subject to different and competing selection pressures that have acted on its immunological and reproductive functions. We suggest that this compromise facilitated development of the bigger brains that enabled archaic and modern humans to migrate out-of-Africa and populate other continents.

Introduction

Comparison of mouse and human genomes has shown that the biggest differences – in terms of both gene content and gene sequence -- occur in genes of the immune and reproductive systems^{1, 2}. This plasticity reflects the vital importance of these two systems and of their genetic diversity in mammalian populations. Working at the interface of defence and reproduction are natural killer (NK) cells, which contribute to innate immunity, adaptive immunity and placentation. Modulating these functions are variable NK-cell receptors that recognize polymorphic MHC class I molecules. Because of the competing and changing needs of their various functions, these receptor–ligand systems evolve rapidly and are inherently unstable. As we discuss, in the course of human history the acquisition of upright walking and bigger brains put limitations on immunity and reproduction, but enabled humans to expand their range out-of-Africa to other continents. The success of these migrations likely required maintenance of a minimal, essential diversity in both polymorphic

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Glossary

[AU: please provide brief (1-2 sentence) definitions for these terms]

Population bottleneck

Adaptive introgression

Simian primates

Prosimian primates

Genetic drift

KIR lineages: I, II, III and V

Balancing selection

MHC class I molecules and variable NK-cell receptors. Loss of diversity, as occurs in population bottlenecks [G], could be compensated by selection for new variants or adaptive introgression [G] of old variants from archaic into modern human populations.

Convergent evolution of variable lymphocyte receptors

All vertebrate and invertebrate animals have an innate immune system, but adaptive immunity is a uniquely vertebrate feature³. Underlying adaptive immunity are variable, clonally distributed lymphocyte receptors that are products of rearranging genes. Such systems have evolved independently in jawed and jawless vertebrates. Almost all extant vertebrates are jawed and have variable B- and T-cell receptors built from immunoglobulin-like domains. In jawless vertebrates -- the lampreys and hagfish -- lymphocytes resembling B cells and T cells have variable receptors constructed from leucine-rich repeats⁴. Although they are structurally unrelated, the variable B- and T-cell receptors of jawed and jawless vertebrates have remarkably parallel functions, which are probably the consequence of convergent evolution driven by universal selection pressures imposed by pathogens.

More prone to convergent evolution are the variable receptors of natural killer (NK) cells, lymphocytes that contribute to both innate and adaptive immunity, and also, in placental mammals, to reproduction^{5, 6}. Whereas the convergence of B- and T-cell receptors is seen only between species that diverged >500 million years ago, convergence of variable NK-cell receptors is apparent among placental mammals that diverged 55-65 million years ago⁷. The receptors in question recognize polymorphic determinants of MHC class I molecules and are the products of diverse families of non-rearranging genes that exhibit allelic polymorphism and gene-content variability⁸⁻¹⁰. During development, NK cells are 'educated' by self-MHC class I molecules to monitor other cells for the quality and quantity of their MHC class I expression¹¹. Unusual MHC class I expression can be a sign of infection, cancer or invading cells from another person, as occurs naturally in pregnancy⁵. Although we focus here on the variable interactions between NK-cell receptors and MHC class I ligands, we must emphasize that this diversifying element to NK-cell function occurs always in the context of conserved interactions between other NK cell receptors and their MHC class I ligands, such as that of human CD94–NKG2A with HLA-E¹².

Variable NK cell receptors are inherently unstable

The variable NK-cell receptors of mouse and human, the species most studied by immunologists, are products of convergent evolution. Whereas immunoglobulin-like domains form the MHC class I-binding site of human killer-cell immunoglobulin-like receptors (KIRs), the binding site of mouse Ly49 receptors is a different type of domain, which resembles that found in calcium-dependent lectins¹³. Emphasizing their independent evolution, KIRs and Ly49 receptors bind non-overlapping sites on the surface of MHC class I molecules⁹ (Figure 1A). Exploratory phylogenetic comparisons have identified a few other species that use Ly49 receptors (rat and horse) or KIRs (simian primates [G] and cattle) as variable NK cell receptors¹⁰. No species is known to diversify both KIRs and Ly49 receptors, but several species diversify neither, preserving KIR and Ly49 as conserved, single-copy genes¹⁴.

Although superficially similar, the cattle and simian primate KIR families are divergent, having arisen from different founder genes — KIR3DX and KIR3DL, respectively¹⁵. These genes arose by duplication of an ancestral KIR gene in a non-placental mammal ~140 million years ago, and were both inherited by placental mammals, where KIR3DX duplicated and diversified in cattle¹⁶, whereas KIR3DL became non-functional. The converse occurred in simian primates, where KIR3DL diversified and KIR3DX became inactive. Prosimian primates [G] lack KIR3DL, and have single KIR3DX and Ly49 genes

and instead have diversified CD94 and NKG2¹⁷, genes that in humans specify the conserved CD94-NKG2 receptors for HLA-E¹⁸ (Figure 1B). That independent evolution of four families of variable NK cell receptors has been revealed by study of <1% of the >4,000 extant species of placental mammal¹⁹ indicates that this phenomenon might have occurred many times during their 132 million-year history⁷.

Implicit to the generation of a new family of variable NK cell receptors is that an older family collapsed, either because its functions were lost or became no longer useful. Several features of the mouse and human systems of variable NK-cell receptors and MHC class I ligands make them vulnerable to such an end. First, because the receptors and their MHC class I ligands are variable and segregate on different chromosomes, in any generation only a fraction of individuals have any given ligand-receptor interaction²⁰. In a population bottleneck [G] with accompanying genetic drift [G], either a ligand or its cognate receptor can be lost from the population, and successive population bottlenecks could have the cumulative effect of eliminating all functional ligand-receptor interactions. Second, polymorphic MHC class I molecules that are ligands for variable NK-cell receptors also present antigens to CD8⁺ T cells, which creates competition between NK-cell and T-cell immunity. Thus, during epidemics of infectious disease, the combination of decreasing population size, genetic drift, and selection for those MHC class I variants offering superior T-cell immunity could have the 'unintended' consequence of eliminating other MHC class I variants that are NK-cell receptor ligands. Third, a similar form of competition could occur between NK-cell functions in immunity and reproduction. An episode of selection exerting pressure on one of these systems could eliminate ligand-receptor interactions that are useful to the other system (see later).

Co-evolution of MHC class I molecules and KIRs in simian primates

Comparison between different mammalian orders has revealed the rapid and convergent evolution of variable NK-cell receptors²¹. A focus on simian primates shows the dynamic co-evolution between MHC class I molecules and KIRs. Because catarrhine (Old World monkeys, apes, and humans) and platyrrhine (New World monkeys) primates have divergent sets of KIR and MHC class I molecules²², to gain perspective on the details of the human system of KIRs our focus can be further narrowed to the catarrhines, species that emerged only 20-38 million years ago²³.

In the human MHC on chromosome 6, polymorphic HLA-A, -B, and -C and conserved HLA-G are the MHC class I genes that encode KIR ligands; we will call their counterparts in other catarrhine species MHC-A, -B, -C and -G. The human KIR gene family is part of the leukocyte receptor complex (LRC) on chromosome 19^{24, 25}. Catarrhines have a common organization to the KIR locus²⁶ and share four phylogenetic lineages of KIR (I, II, III and V [G]) that are distinguished by their structure and specificity for MHC class I²⁷. In humans, the most thoroughly studied system (Figure 2), HLA-G is recognized by a lineage I KIR²⁸, whereas four, mutually exclusive, epitopes of polymorphic HLA class I are recognized by lineage II and III KIRs. (A ligand for lineage V KIRs has yet to be identified.) Lineage II KIRs recognize the HLA-A epitope A3/11 and the Bw4 epitope of HLA-A and -B, whereas lineage III KIRs recognize the C1 (HLA-C1) and C2 (HLA-C2) epitopes of HLA-C and two HLA-B allotypes that also carry the C1 epitope²⁹. Knowledge of the KIRs and MHC class I molecules of non-human catarrhines has given insight to the order in which key components of the human system evolved, and demonstrates how the KIR locus has co-evolved to keep up with the changes in MHC class I genes.

Going back in phylogeny, Old World monkeys are the most divergent species to have counterparts to human KIR ligands. In macaques, a multiplicity of MHC-A and MHC-B genes^{30,31} and presence of the Bw4 sequence motif³² are associated with a corresponding

multiplicity of lineage II KIRs³³⁻³⁵. Macaques lack MHC-C, and correspondingly have just one lineage III KIR, which may not recognize MHC class I. Orangutan has many fewer MHC-A and -B genes than macaques and only one lineage II KIR. These reductions correlate with the emergence of MHC-C and an increased number of lineage III KIRs in orangutan²⁷. The extent of the changes is impressive, because only ~50% of orangutan MHC haplotypes have the MHC-C gene, and its constituent allotypes carry only HLA-C1. Because some orangutan, chimpanzee and human MHC-B allotypes carry the C1 epitope, and MHC-C evolved from an ancestral MHC-B gene, the C1 epitope most likely arose at an MHC-B gene and was a feature of the gene that differentiated to become MHC-C³⁶. Thus the C1 epitope functioned as a KIR ligand for several million years before emergence of the C2 epitope and fixation of MHC-C in a common ancestor of humans and chimpanzees³⁷. Accompanying these events was a further increase in the number of lineage III KIRs: chimpanzees having nine lineage III KIRs and one lineage II KIR, humans having seven lineage III KIRs and two lineage II KIRs³⁶.

Elaboration of the interactions between MHC-C molecules and lineage III KIRs reached its peak with chimpanzees, which have eight MHC-C-specific KIRs, including inhibitory and activating receptors for HLA-C1 and HLA-C2³⁸ (Figure 3A). By contrast, humans have only three KIRs specific for HLA-C: an inhibitory C1 receptor (KIR2DL2/3), an inhibitory C2 receptor (KIR2DL1) and an activating C2 receptor (KIR2DS1). In addition, the activating receptor KIR2DS4, which is the only lineage III KIR common to humans and chimpanzees, recognizes some C1- and C2-bearing HLA-C allotypes, as well as the A3/11 epitope of HLA-A*11³⁹. Three other expressed human activating lineage III KIRs (KIR2DS2, KIR2DS3 and KIR2DS5) once recognized HLA-C, but during the course of human evolution they acquired mutations that blocked this function⁴⁰⁻⁴². Finally, human-specific KIR2DP1 is a completely inactivated gene that once encoded an inhibitory C1 receptor. In contrast to the human situation, none of the chimpanzee lineage III KIRs has lost the capacity to recognize MHC class I (Figure 3A).

Further distinguishing the human and chimpanzee systems is the distribution of the C1 epitope³⁶. Approximately 25% of chimpanzee MHC-B variants retain the C1 epitope and function as ligands for C1-specific KIRs, whereas human C1 is almost exclusively associated with HLA-C (Figure 3B). An obvious benefit to MHC-C becoming a specialized KIR ligand is the relaxation of pressure on KIRs to keep up with changes in MHC-A and -B driven by CD8⁺ T cells.

The KIR locus in gibbons (the lesser apes) does not conform to the progression observed in other catarrhines, where a multiplicity of lineage II KIRs was replaced by a multiplicity of lineage III KIRs with the emergence and elaboration of MHC-C. In gibbons, the KIR locus has been subject to intensive deletion and mutation, causing loss of structural integrity⁴³. Targets for inactivation were KIR lineages I and III, which in humans recognize HLA-G and HLA-C, respectively. Corresponding to these lost KIRs, the gibbon MHC lacks the genomic segments that carry MHC-G and MHC-C in hominids³¹. This decline of gibbon KIR and MHC class I vividly captures the transient nature and species specificity of variable NK cell receptors.

Human evolution of two balanced KIR haplotype groups

Crucial innovations in hominid evolution were the emergence of MHC-C and establishment of its C1 and C2 epitopes as dominant KIR ligands, present in all human populations and maintained by balancing selection [G]. Subsequently, and only during human evolution, the KIR haplotypes became divided into two groups, A and B, with qualitatively different KIR gene content^{26, 44}. The A haplotypes, comprising mainly inhibitory KIR that recognize HLA class I, are more similar to chimpanzee KIR haplotypes³⁶. Uniquely human are the B

haplotypes, which have accumulated genes encoding KIRs that have decreased or no binding to HLA class I molecules, such as KIR2DS2, KIR2DS3 and KIR2DS5 mentioned previously^{45, 46} (Box 1). The differences between A and B KIR haplotypes evolved in two stages. The first stage, involving the centromeric part of the haplotype, occurred soon after the human and chimpanzee lines diverged ~6-7 million years ago. The telomeric half of the B haplotype evolved at a later time, ~1.7 million years ago⁴⁶, contemporaneous with emergence of the genus *Homo* and of *Homo erectus*, the first ancestral human to achieve fully upright walking and successful migration out-of-Africa to populate Eurasia^{47, 48}.

KIR A and B haplotypes are present in all human populations⁴⁹ and maintained by balancing selection⁵⁰. This implies that the haplotype groups have to some extent become specialized for distinctive and complementary functions. Several lines of evidence point to these differences being associated with the involvement of NK cells in both immunity and reproduction.

KIR interactions with HLA-C influence reproduction

Whereas HLA-A, -B and -C are co-expressed by most cells of the body, a crucial difference occurs in pregnancy, when extra-villous trophoblast (EVT) cells express HLA-C but neither HLA-A nor HLA-B^{51, 52}. EVT cells are fetal cells that invade the mother's uterus, where they transform the spiral arteries into large vessels capable of conducting sufficient blood to the placenta until the end of pregnancy⁵³ (Figure 4). The extent of arterial transformation by trophoblast affects the success of reproduction. Defective conversion by EVT cells, resulting in insufficient blood supply to the placenta, can lead to pre-eclampsia, stillbirth or low birth weight. Neither should babies become too big, because this can lead to damaging and even fatal complications in childbirth. Trophoblast invasion must therefore be controlled. This is the likely function of uterine NK cells, which dominate the uterine leukocyte population⁵⁴⁻⁵⁸ and preferentially express HLA-C-specific KIRs⁵⁹.

Contact, communication and co-operation between EVT cells and uterine NK cells could involve recognition of HLA-C, -E, and -G on EVT cells by their cognate uterine NK-cell receptors. Of these interactions, which involve both activating and inhibitory receptors, only that between HLA-C and KIRs is polymorphic and influences the course of pregnancy in a genetically determined manner⁶⁰. At highest risk for recurrent miscarriage, pre-eclampsia and fetal growth restriction -- syndromes that are associated with an insufficiently invasive placenta -- are mothers homozygous for KIR haplotype A and HLA-C1 carrying a fetus with HLA-C2 inherited from the father⁶¹⁻⁶³. In this combination the mother has the inhibitory C2 receptor (KIR2DL1) but not its activating counterpart (KIR2DS1). Thus inhibition of uterine NK cells, mediated by KIR2DL1 recognition of HLA-C2, is implied as a mechanism causing insufficient uterine invasion by EVT cells. In this model, the inhibited NK cells would provide insufficient help, by way of cytokines and cell-cell contacts, to the EVT cells.

Women can be protected against these pregnancy disorders by KIR B haplotypes, particularly the telomeric region⁶¹. Among the KIR encoded by telomeric B is KIR2DS1, the activating C2 receptor which can counter the inhibitory effects of KIR2DL1 (Box 1). The centromeric region of B haplotypes can also contribute to protection: some B haplotypes lack the KIR2DL1 gene (Box1) and the others have alleles encoding weak forms of KIR2DL1. Thus 2DL1*003, the common allele of the A haplotype has stronger signaling function⁴⁵ and higher avidity for C2 (H. Hilton and P. Parham unpublished observations) than 2DL1*004, the common B haplotype allele⁴⁶. In addition, KIR2DL3, the inhibitory C1 receptor of the A haplotype, is replaced by the combination of inhibitory KIR2DL2, a stronger C1 receptor that crossreacts with C2^{29, 42}, and activating KIR2DS2, which cannot recognize HLA class I molecules. Together KIR2DS2 and KIR2DL2 act to decrease the

frequency of NK cells expressing KIR2DL1, a competitive effect that could arise because KIR2DL2, like KIR2DL1, recognizes HLA-C2 and is expressed before KIR2DL1 during NK-cell differentiation and education⁶⁴. Thus, by a variety of mechanisms, the B haplotype KIRs diminish the influence of the strong KIR2DL1 forms carried by A haplotypes and that are associated with pregnancy syndromes such as pre-eclampsia.

Obstructed labor, caused by babies too big to pass through the birth canal, is another potentially fatal complication of pregnancy. Having very large babies (>95th percentile) correlates with women who have telomeric region B haplotype genes, and thus KIR2DS, and a fetus expressing paternally-derived C2⁶⁵ (A. Moffett, unpublished observations). The implication being that in these pregnancies KIR2DS1-mediated NK-cell activation gives too much help to EVT cells and promotes overly deep placental invasion. Thus KIR B haplotypes can confer both advantage and disadvantage in pregnancy; this is also the case for the A haplotypes, because of the allelic relationship between the two haplotype groups. Such correlations are consistent with the human KIR A and B haplotype dichotomy having evolved under selective pressure from reproduction. Evidence for this selection are worldwide inverse correlations between HLA-C2 and KIR A haplotype frequencies⁶³ and between HLA-C1 and KIR B haplotype frequencies⁶⁶. This situation reduces the frequency of pregnancies at risk for pre-eclampsia, when KIR A homozygous mothers bear HLA-C2⁺ babies.

Immunity and reproduction set KIR haplotype balance

Human KIR A haplotypes combine fixed gene content with receptors having a high degree of polymorphism, which modulates functional recognition of HLA-A, -B and -C epitopes and distinguishes human populations. These are characteristics of immune-system molecules that detect pathogens and become subject to their strong selection pressures. Consistent with this thesis, the genotype correlated with resistance to acute hepatitis C virus (HCV) infection is homozygous KIR A haplotype and HLA-C1^{67, 68}. This is a stringent test, because HCV is a virus against which most human immune systems are ineffective. A minority (20-30%) of individuals terminate acute HCV infection, whereas the majority develop chronic infection and increased risk of liver cancer⁶⁹. KIR A haplotype homozygosity is also associated with resistance to acute Ebola-virus infection⁷⁰ and, in combination with HLA-C1 homozygosity, a favorable response to treatment for chronic HCV infection. Although few acute infections have been studied for KIR association, the results suggest that KIR A haplotypes can provide more effective immunity against acute viral infections than KIR B haplotypes, with their accumulation of attenuated and less polymorphic KIRs.

That homozygosity for HLA-C1 and KIR A haplotype is associated with both resistance to HCV infection and susceptibility to pre-eclampsia can be seen as evidence for the compromise made, during evolution of the A and B KIR haplotypes, between the functions that NK cells exert in immunity and reproduction. Illustrating how this might have worked is the cyclical model shown in Figure 5A. When epidemic infection passed through a population, causing disease, death (particularly of the young) and social disruption, selection favored KIR A over B haplotypes and HLA-C1 over HLA-C2. When the epidemic subsided, the surviving and now smaller population was immune to further infection and enriched for KIR A haplotypes and HLA-C1, with corresponding reduction of KIR B haplotypes and HLA-C2 epitopes. At this juncture, survival of the current generation was no longer the issue, and the priority became production of the next generation. In this second phase of the cycle, selection favored KIR B haplotypes and HLA-C2, factors that enhance the generation of larger and more robust progeny. Because human history has always involved successive cycles of the kind shown in Figure 5, this alternating pattern of selection pressures has been persistent. That all extant human populations maintain significant frequencies shows that KIR A and KIR B haplotypes (Figure 5B), as well as HLA-C1 and HLA-C2, have all been

necessary for the survival of human populations, although none of them is essential for the health and survival of individuals.

Bigger brains and the emergence of KIR B haplotypes

Since human and chimpanzee ancestors separated 6-7 million years ago, chimpanzees diverged less from the common ancestor while remaining in equatorial Africa^{48, 71, 72}, whereas humans diverged further, while expanding their range throughout the world's landmass⁷³. The emergence of KIR B haplotypes was a part of this human-specific evolution and possibly reflects changes in locomotion, anatomy and reproduction. That chimpanzees did not undergo such changes could explain why their KIR locus was not selected to form two haplotype groups as a means of improving reproductive success.

A key human innovation was bipedalism. Whereas chimpanzees mainly use all four limbs to knuckle walk, adult humans walk fully upright on two legs. This development, which was largely achieved by 3.66 million years ago⁷⁴, required considerable anatomical changes that altered the size and shape of the human female pelvis and the dimensions of the birth canal. When first evolved these pelvic changes did not affect obstetric mechanics, but with the evolution of increasingly bigger brains problems began to arise. The size of the human baby's head increased until it reached the limit defined by the birth canal. Thus at term a modern human baby's head just fits into the birth canal, whereas chimpanzee babies have the luxury of more headroom. In the course of human evolution, birthing became a difficult, dangerous and often fatal business, requiring rotation of the baby to pass through the pelvis and obligatory assistance from a midwife⁷⁵⁻⁷⁷.

The development of bigger brains required more nourishment in utero, putting greater demands on the blood supply to the placenta^{78, 79}. This was achieved by increasing the extent of the placental invasion of the uterine arteries, the process mediated by EVT and regulated by uterine NK cells. Pro-simians have a non-invasive placenta, whereas gibbons and Old World monkeys modify their arteries by trophoblast moving down the inside of the arterial wall from the placenta. This also occurs in hominids, but in addition trophoblast cells invade through the stroma to go deep into the myometrium, where they surround and destroy the medial smooth muscle, reconstructing the arteries with fetal cells⁸⁰. This deep invasion correlates with the emergence of MHC-C and cognate KIRs and their role in the regulation of EVT cells by uterine NK cells. That MHC-C1 and activating and inhibitory C1-specific KIRs evolved first (before MHC-C2 and C2-specific KIRs) suggests that these interactions contributed to deeper placental invasion in ancestral hominids. Subsequently, in a common ancestor of humans and African apes, increasing brain size and associated risks of death during childbirth, favored the evolution of MHC-C2 and both activating and inhibitory C2-specific KIRs. The resulting bipartite system strikes a balance that reduces the likelihood of too little or too much invasion.

At term, the human brain is about twice the size of the chimpanzee brain⁸¹. Most of this increase in human brain size occurred gradually over the last 2 million years, a progression seen in specimens of *Homo erectus*^{47, 48}, and correlating with emergence of the telomeric part of the KIR B haplotype ~1.7 million years ago⁴⁶. A key component of this telomeric part, activating C2-specific KIR2DS1, is seen today to protect against recurrent abortion, pre-eclampsia and low birth weight, but at earlier times in human evolution, when the heads of human babies were smaller than the birth canal, it could have helped increase human brain size by increasing placental invasion through NK-cell mediated activation of trophoblast. Consistent with this mechanism, KIR2DS1 is over-represented among mothers who have large babies causing obstructed labor (A. Moffett, unpublished observations). The centromeric part of the KIR B haplotype emerged before the telomeric part, soon after the human and chimpanzee lines diverged 5-7 million years ago⁴⁶. That the protection against

recurrent abortion, pre-eclampsia and low birth weight provided by the centromeric part of the KIR B haplotype is less than that provided by the telomeric part correlates with the smaller increase in brain size that occurred in the human line of australopithecines prior to emergence of the genus *Homo*⁴⁸.

Keeping HLA and KIR diversity during human migrations

Upright walking and an increasingly bigger brain contributed to the success of *Homo erectus* in extending its range out-of-Africa and populating Eurasia from ~1.8-0.3 million years ago. Similar migration and colonization has occurred on at least two other occasions since. The first, ~600 thousand years ago, was by ancestors of the neandertals (*Homo neanderthalensis*), the latter emerging in Europe ~300 thousand years ago and surviving in Eurasia until ~30 thousand years ago⁸². The second occurred ~67 thousand years ago by anatomically modern humans (*Homo sapiens*) who had emerged in Africa ~200 thousand years ago⁸³. After reaching Eurasia they outlived the archaic human populations and went on to colonize much of the earth's landmass⁸⁴.

In present-day human populations, genetic diversity decreases with the distance of migration from Africa⁸⁵. This steady loss of diversity is the consequence of passage through a succession of population bottlenecks as migrant groups settled into new territories and expanded their populations. With population growth came competition for resources and resulting conflict that could split the population: one part staying put, the other forced out to continue migration^{73, 86}. Both factions emerging from such episodes could have less genetic diversity than when they were combined. Other genetic bottlenecks would accompany periods of hardship, with decrease in population size owing to infection, famine, drought and warfare -- either individually or in combination, as has often occurred.

HLA class I molecules and KIRs are encoded by diverse, polymorphic gene families that by diversifying the immune systems of individuals within a population increase the probability that the population will survive the successive epidemics of infectious disease caused by diverse and rapidly evolving pathogens. For such genes, which have no wildtype, the effects of population bottlenecks are potentially catastrophic, because variants not under immediate selection can be irretrievably lost. This has raised an important question: how much diversity of HLA class I molecules and KIRs has been necessary for human populations to survive? Possible answers to this question can be obtained by studying present-day indigenous populations.

The Americas were the last continents to be populated by modern humans; by Asian migrants from Siberia who arrived in Alaska ~17,000 years ago and then extended their range by southward migration throughout North and South America⁸⁷. Genome-wide, South Amerindian populations have the least genetic diversity of all modern human populations, as exemplified by their loss of polymorphism at the ABO system of blood group antigens⁸⁸. Although HLA class I diversity also decreases to some extent with distance from Africa, substantial diversity is retained by South Amerindian populations⁸⁹⁻⁹¹: some 4-6 allotypes of HLA-A, -B and -C, including ones that carry the C1, C2 and Bw4 epitopes recognized by KIRs. Replenishing lost diversity were new variant alleles, particularly HLA-B alleles, that were derived by recombination between the alleles that were brought from Siberia⁹²⁻⁹⁴ (Figure 6A). The KIR genes of South Amerindians present a similar picture. With the exception of KIR2DS3 in some tribes, such as the Yucpa (Box 1), South Amerindians retain all the major KIR genes found worldwide, as well as a balance between A and B KIR haplotypes, and several alleles, including some new variant alleles, at the polymorphic KIR genes. That such sets of KIRs and HLA class I molecules were maintained despite numerous population bottlenecks suggests they represent the minimal diversity necessary for human populations to survive over periods >10,000 years. The corollary being that any population

lacking such diversity either died out or was assimilated into another population. Notably, the HLA-A3/11 epitope recognized by KIRs was not necessary for the survival of Amerindians (Figure 2).

Contribution of archaic HLA to migrating modern humans

When modern humans first populated the Americas, their only source of additional HLA class I and KIR diversity was new variant alleles formed by point mutation or recombination. For the modern humans who migrated out-of-Africa ~67 thousand years ago another potential source of genetic diversity was the existing Eurasian populations of archaic humans, notably the neandertals, with whom they overlapped for ~30 thousand years^{95, 96}. Whole-genome comparisons show that modern humans did indeed meet and mate with archaic humans, giving rise to viable offspring who passed their neandertal genes on to their modern human descendants in Eurasia, a process known as introgression. The overall neandertal contribution to present-day Eurasian genomes is thus estimated to be 1-4%⁹⁷. The neandertal woman whose genome has been sequenced was heterozygous for HLA-A, -B, and -C and carried alleles that are identical to common alleles in the present-day Eurasian population. Her HLA type includes the C1, C2 and Bw4 epitopes, but not the A3/11 epitope. Within the present-day population, the neandertal's two HLA haplotypes and some of the alleles are found to be present in Eurasia but absent from Africa. Factors having this geographic distribution, for example C1-bearing HLA-C*07:02 and C2-bearing HLA-C*16:01, are unlikely to have come out-of-Africa with migrating modern humans and are more likely to have been acquired by them in Eurasia by introgression from neandertals. Importantly, the frequencies of introgressed HLA class I alleles are much greater than the 1-4% genome average for introgressed neandertal genes, indicating that they were functionally beneficial and driven to higher frequency by natural selection, a process known as adaptive introgression. This would have been a much more efficient process for migrant modern humans to replenish lost HLA class I diversity, including lost ligands for KIRs, than selecting for new recombinants and point mutants, because one mating could give a full set of new and distinctive HLA-A, -B, and -C allotypes that had already stood the test of time in a human population in the Eurasian environment. For example, HLA-B*73 is an unusually divergent HLA-B allele of archaic origin that entered the modern human population in western Asia (Figure 6B). Whereas the new variants that evolved in South America differed at 1-3 amino acid substitutions, the hypothetical acquisition of HLA-B*73 by these populations would have given them a new variant with 41 substitutions, at 16 positions of functional importance (Figure 6A).

The genome sequence of a Denisova hominin suggests this recently discovered type of archaic human also overlapped with modern humans in a broad range from Siberia to Southeast Asia⁹⁸. In present-day genomes, the Denisovan contribution is highest in Melanesia, where it reaches 4-6%⁹⁸. The Denisovan woman whose genome has been sequenced was heterozygous for HLA-A, -B and -C and carried HLA-A and HLA-C alleles that are common in present-day South-east Asian and Melanesian populations. By contrast, her HLA-B alleles are rare or absent from present-day populations, but are recombinants of common present-day HLA-B alleles. Included in the Denisovan HLA type are all four epitopes recognized by KIRs: C1, C2, Bw4 and A3/11. Within the present-day population, the Denisovan's two HLA haplotypes and some of the alleles are present in Southeast Asia and Melanesia, but absent from Africa. Moreover, their frequencies are indicative of adaptive introgression. Notably HLA-A*11 reaches frequencies of 64% in Melanesia. At HLA-A, for which the data are more comprehensive, the distribution and linkage disequilibrium in present-day populations estimate the contribution of archaic HLA-A to non-Africans population to be 50-95%. The unexpected extent of this adaptive introgression raises the fascinating possibility that acquisition of polymorphic immune-system and

reproductive-system genes from archaic humans was necessary for the survival and success of some modern human populations outside of Africa.

Concluding remarks

In this article we have drawn on observations from the fields of immunology, genetics, reproduction, anthropology and comparative anatomy to give a speculative working model that can explain the contribution of variable NK cell receptors and MHC class I ligands to human evolution and why they evolved to be different from their counterparts in other hominid species. Functional interactions between polymorphic MHC class I molecules and variable $\alpha\beta$ T-cell receptors have been maintained for >500 million years. By contrast, systems of variable NK cell receptors that recognize polymorphic MHC class I molecules are inherently unstable, are shorter lived, and have evolved by convergence in placental mammals on several occasions. Underlying this instability are the competing demands of the functions that NK cells carry out in innate immunity, adaptive immunity and placental reproduction. The human KIR system of variable NK cell receptors has counterparts only in the simian primates, and exhibits extraordinary inter-species and intra-species variation. KIRs co-evolved with MHC-A, -B, -C and -G in the catarrhine primates, with the emergence in hominids of MHC-C as a major source of polymorphic KIR ligands and the only ones expressed by extravillous trophoblast cells. During formation of the placenta these fetal cells invade the uterus to remodel maternal vessels that will supply the placenta with blood and nourish the fetus throughout the pregnancy. This process appears controlled by cooperative interactions between fetal trophoblast and maternal NK cells in the uterus, and diversified within hominid populations by polymorphic interactions between trophoblast MHC-C and KIR on uterine NK cells. Increasing brain size in hominid evolution necessitated deeper invasion of the uterus in pregnancy and increased variation in MHC-C and cognate KIRs. Unique to human evolution was acquisition of upright walking, which facilitated migrations out-of-Africa, and further increases in brain size. Also associated with these developments, and unique to human species, was the formation of two groups of KIR haplotype. These complementary A and B KIR haplotypes are maintained in all human populations and seem to represent a historical compromise between the immunological and reproductive functions of NK cells, that was driven by selective pressure on the nervous system for bigger and better brains. Migration of modern human populations out-of-Africa to populate other continents was associated with maintenance of a minimal, essential set of KIR and HLA class I variants. One way that modern humans replenished the diversity lost in population bottlenecks was selection of new variants arising *de novo*; another and possibly more effective mechanism was to acquire old variants through reproduction with archaic humans.

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Box 1: KIR A and B haplotypes

Human KIR genes are evenly distributed between the centromeric and telomeric regions. Both regions have alternative and distinctive gene-content motifs, exemplified here by the Yucpa, a South Amerindian population subject to strong selection by infectious disease and population bottleneck⁵⁰. Combination of the centromeric A1 and telomeric A1 motifs forms the KIR A haplotype, which encodes inhibitory HLA-C1 (KIR2DL3) and HLA-C2 (KIR2DL1) receptors in the centromeric region and inhibitory HLA-Bw4 (KIR3DL1) and HLA-A3/11 (KIR3DL2) receptors in the telomeric region. KIR2DS4, which recognizes HLA-A3/11 and some C1- and C2-containing HLA-C, is the activating receptor of the A haplotype. A haplotypes have identical KIR gene content, but vary by allelic polymorphism. For each gene colours denote different alleles: the most frequent allele being red, the next most frequent yellow, and so on to green, blue and purple. Divergent allelic lineages for KIR2DL2/3 (*L2* and *L3*), KIR3DL1/S1 (*L1* and *S1*) and KIR2DS4 (full length [*fl*] and deletion [*del*] forms) are indicated: neither KIR3DS1 nor KIR2DS4 *del* bind HLA class I. The Yucpa have five different A haplotypes (A1-A5), comprising 46.2 % of the total KIR haplotypes. At similar frequency (47.5%), the KIR B1 haplotype comprising centromeric B2 and telomeric B1 motifs has no allelic polymorphism. This haplotype lacks genes for inhibitory HLA-C2 and HLA-Bw4 receptors and has a distinctive form (KIR2DL2) of the inhibitory C1 receptor that crossreacts with C2. Further distinguishing KIR B1 from KIR A haplotypes is the activating HLA-C2 receptor (KIR2DS1), and five expressed KIRs that cannot recognize HLA class I: inhibitory KIR2DL5 and activating KIR2DS2, KIR3DS1, KIR2DS5 and KIR3DS1. A repetitive sequence between KIR3DP1 and KIR2DL4 facilitates recombination of centromeric and telomeric motifs, yielding haplotypes having centromeric A with telomeric B motifs (haplotype B2) or centromeric B with telomeric A motifs (haplotypes B3 and B4). Although they are at low frequency in the Yucpa, such recombinants are more frequent in some other populations. Because centromeric B and telomeric B motifs dominate in studies of disease association, all haplotypes having centromeric B or telomeric B or both centromeric and telomeric B are grouped together as KIR B haplotypes.

A

B

Figure 1. Convergent evolution of variable NK-cell receptors for MHC class I

Panel A shows how human KIRs and mouse Ly49 lectin-like receptors bind to different and non-overlapping sites on the surface of the MHC class I molecule. KIRs interact with the upward face of the MHC class I molecule formed by the helices of the α_1 and α_2 domains and the peptide bound in the groove between them⁹⁹. By contrast, Ly49 binds underneath the peptide-binding groove, where it interacts with all four domains of the MHC class I molecule: α_1 , α_2 , α_3 , and β_2 -microglobulin (β_2 -m)⁹. Panel B shows how human KIRs and the human lectin-like receptor CD94:NKG2A bind to overlapping sites on the surface of the MHC class I molecule. The ribbon diagram shows the upward face of the MHC class I molecule with the space-filling image of the peptide between the helices of the α_1 and α_2 domains. The dashed ellipses and coloring of the ribbon diagram denote the areas to which the KIRs (red) and CD94:NKG2A (green) bind. The overlap of the binding sites is colored yellow. Being a lectin-like receptor, CD94:NKG2A is structurally more similar to mouse Ly49 than to human KIRs, but it binds to a different site on MHC class I than does Ly49^{100, 101}. The overlap in the binding sites for KIRs and CD94:NKG2A on HLA class I molecules does not cause competition between the two receptors, because CD94:NKG2A is

restricted to interaction with HLA-E, whereas KIRs are restricted to interactions with HLA-A, -B, -C and -G. On the contrary, CD94:NKG2A and KIRs have complementary roles in NK cell biology because the interaction of HLA-E with CD94:NKG2A is highly conserved, whereas the interactions between KIRs and HLA-A, -B, and -C are highly diverse.

Figure 2. Humans KIRs recognize four epitopes of HLA-A, -B and -C

a | The pie charts show the distribution of the four target epitopes (green, yellow, blue and red) in five human population groups. These four epitopes are mutually exclusive, so that each HLA-A, -B and -C allotype can either have one of these epitopes or is not a ligand for KIR (grey). The C1 epitope (colored blue) is carried by HLA-C allotypes with asparagine at position 80 and certain Asian HLA-B alleles that have asparagine at position 80 and valine at position 76. The C2 epitope (colored red) is carried by HLA-C allotypes that have lysine at position 80. The Bw4 epitope (colored green) is carried by HLA-A and -B allotypes that have arginine at position 83. The A3/11 epitope (colored yellow) is carried by two HLA-A allotypes, HLA-A*03 and HLA-A*11, and seems to be as peptide-dependent as the $\alpha\beta$ T-

cell receptor¹⁰². Data are pooled from a minimum of eight populations in a group up to a maximum of fifty five. b | The upper linear diagram shows the order of the 15 human KIR genes in the KIR locus on human chromosome 19 and the phylogenetic lineages to which they belong (I, II, III or V). Beneath the gene boxes are shown the HLA class I specificities of the encoded receptors. The smaller point size denotes a receptor that only recognizes some of the allotypes carrying the epitope. The boxes for genes encoding inhibitory receptors are coloured orange, the boxes for genes encoding activating receptors are coloured light green and boxes corresponding to pseudogenes are shaded gray. The lower linear diagram shows the framework genes and the centromeric and telomeric regions of gene-content variability that they flank and define.

A

Figure 3. Co-evolution of HLA-C and KIR lineage III in hominids
a | The organization and KIR-gene content of orangutan²⁷, chimpanzee³⁶ and human⁴⁶ KIR haplotypes are compared. Boxes representing the framework genes, which are common to all haplotypes, are shaded dark gray; boxes representing variable lineage I and II KIR genes are shaded light gray, and variable lineage III genes are shaded according to species: orange (orangutan), green (chimpanzee) and purple (human). For those lineage III KIRs that recognize MHC class I, the epitope specificities are given in the gene box, with white script for activating KIRs and black script for inhibitory KIRs. With the exception of KIR2DS4 (the boxes labeled A,C) , which is present in humans and chimpanzees, all of the variable lineage III KIR genes are species-specific. b | The pie charts compare the distribution of the four MHC class I epitopes recognized by KIRs in chimpanzees and humans. The Bw4 (colored green) epitope originated at MHC-B. In chimpanzees Bw4 is only carried by MHC-B allotypes, whereas in humans it was also transferred to MHC-A. The C1 epitope (colored blue) also originated at MHC-B and was directly inherited by MHC-C. Whereas chimpanzees retain C1 at both MHC-B and MHC-C, in humans the C1 epitope has been largely eliminated from HLA-B. The C2 epitope (colored red) originated with MHC-C and remains exclusively an epitope of MHC-C in chimpanzees and humans. The A3/11 epitope (colored yellow) is specific to the human HLA-A*03 and HLA-A*11 allotypes, has not been correlated with polymorphisms in the HLA-A sequence, and seems to be highly peptide dependent¹⁰².

Implantation	Lemur	Rhesus macaque	Human
<p>uterine epithelium</p> <p>blastocyst</p> <p>endometrium</p> <p>myometrium</p> <p>maternal blood vessel</p>
Blastocyst implants into the uterine epithelium	Trophoblast does not penetrate through the uterine epithelium NK cells are not present	Trophoblast breaches the uterine epithelium and invades the lumen of maternal blood vessels to replace the endothelium and enlarge the vessels some NK cells are present	Greater enlargement of maternal blood vessels is achieved by deeper invasion of trophoblast and invasion of both the insides (endothelium) and outsides (smooth muscle) of the blood vessels. Numerous NK cells are present

Figure 4. Increased invasion of the uterus in primates is associated with the presence of NK cells
 Panel A shows implantation of the blastocyst, an early stage in embryo development, into the uterine epithelium. Co-operative interactions between fetal trophoblast and maternal cells then form the placenta. In prosimians, such as the lemur shown in panel B, the trophoblast cells abut the surface epithelium of the uterus but do not invade. Neither are NK cells present. Nutrients are transferred to the fetus from maternal blood vessels close to the uterine epithelium and in glandular secretions. This arrangement is called an epitheliochorial placenta. The endometrium does not transform into decidua and NK cells are absent. In Old World monkeys, such as the rhesus macaque, trophoblast cells penetrate through the epithelium and invade maternal arteries where the trophoblast cells replace the vascular endothelial cells. This transformation increases the blood supply to the placenta, where nutrients are transferred directly from maternal blood to the fetal capillaries. Accompanying these changes is the presence of NK cells in the decidua, the name given to the endometrium that has differentiated under the influence of progesterone. This type of placenta, which is also seen in human pregnancy, is called a haemochorial placenta. In human placentation, panel D, trophoblast cells invade the blood vessels as in rhesus macaque, but replace the vascular endothelium to a greater degree that extends into the myometrium. In addition, trophoblast cells invade the decidua, replacing the medial smooth muscle with fibrinoid material. Accompanying these changes is the presence of numerous NK cells.

Figure 5. Model for the maintenance KIR A and KIR B haplotypes and HLA-C1 and HLA-C2 epitopes in human populations
a | A hypothetical cycle in which the size of a population changes over time and circumstance. With the onset of an epidemic of an acute and lethal viral infection the population size will progressively decrease, disproportionately so for infants and the younger generation. If the combination of KIR A haplotypes and HLA-C1 epitopes provides resistance to infection, then the surviving population, which will be largely immune to further infection, will have increased frequencies of KIR A and HLA-C1, compared with the starting population, and decreased frequencies of KIR B and HLA-C2. With the end of the infectious cycle the challenge becomes one of reproduction, as the population can only survive if the next generation is sufficiently viable and numerous. In this situation, where there is a strong element of competition among the survivors, there will be selection for the

combination of KIR B haplotypes and HLA-C2, the most recently evolved elements of the system of interactions between KIRs and HLA class I. Pregnancies in which the mother has KIR B and the fetus has C2 are predicted to favor larger and more robust progeny. Thus in this part of the cycle, the frequencies of KIR B haplotypes and HLA-C2 will increase while those of KIR A haplotypes and HLA-C1 will decrease. b | All human populations retain KIR A (red) and KIR B (green) haplotypes, but their relative frequencies vary.

Domain	α_1			α_2										Potential Donor Alleles				
Function	<i>p</i>	<i>t</i>	<i>p</i>		<i>p</i>	<i>p</i>		<i>p</i>	<i>p</i>	<i>pt</i>	<i>p</i>	<i>pt</i>	<i>p</i>					
Position	45	62	67	94	95	97	103	114	116	152	156	163	171	B*15:01	B*39:01	B*40:02	B*48:01	B*51:01
B*35:01	T	N	F	I	I	R	L	D	S	V	L	L	Y					
B*35:04	.	.	.	.	.	.	.	N	Y	.	.	.	.					
B*35:05	.	.	.	T	L	S	.	.	.	.	.	.	.					
B*35:06	.	.	.	.	.	.	.	N	F	.	.	.	.					
B*35:08	.	.	.	.	.	.	.	.	.	.	R	.	.					
B*35:10	.	E	.	.	.	.	.	.	.	.	.	.	.					
B*35:11	.	.	.	.	.	.	.	.	.	E	.	.	.					
B*35:12	.	.	.	.	.	.	V	N	Y	.	.	.	.					
B*35:14	.	.	.	.	.	.	.	.	.	E	W	.	.					
B*35:15	.	.	.	.	.	.	.	.	.	.	.	E	.					
B*35:16	.	E	.	.	.	S	V	.	.	.	.	.	.					
B*35:17	.	.	.	.	.	S	V	.	.	.	.	.	.					
B*35:19	K	.	.	.	.	.	.	.	.	.	.	.	.					
B*35:20	.	.	S	.	.	.	.	.	.	.	.	.	.					
B*35:21	.	.	.	.	.	.	.	.	.	E	.	.	H					

A

Domain	signal sequence					α_1										α_2										α_3					stem/TM/CYT											
Function									p	p	p	tk	pt		pt	tk	p			p	p		p	p	p		pt	p	I													
Position	-23	-21	-11	-10	-8	9	11	12	24	45	67	69	70	71	74	76	77	90	94	95	97	103	113	114	116	131	163	171	194	239	253	267	268	270	275	295	297	299	ins	301	306	
HLA-B*35:01	R	T	W	G	V	Y	A	M	A	T	F	T	N	T	Y	E	S	A	I	I	R	L	H	D	S	S	L	Y	V	P	R	E	P	K	L	E	A	V	I		G	T
HLA-B*73:01	L	M	S	A	L	H	S	V	T	E	C	A	K	A	D	V	G	D	T	W	T	M	L		N	F	R	E	H	I	G	Q	Q	E	C	K	V	T	A	V	V	A

Figure 6. Maintaining HLA diversity during migrations that increased the geographical range of human species

a | Humans first entered the Americas at Alaska after migration from Asia ~17,000 years ago. North, Central and South America were then colonized by southward migration. For present-day Native American populations of North America, the HLA-B alleles largely remain identical to the ones that came with the migrants from Asia. Starting in the southwestern part of the USA and increasing with distance southwards, the Amerindian populations have ‘new’ recombinant HLA-B alleles in which a short sequence segment in one Asian founder allele was replaced by the orthologous segment from another founder allele. This phenomenon is illustrated for the HLA-B*35:01 Asian allele⁹². Shown are fourteen recombinants of HLA-B*35:01, the amino-acid positions that were affected by the recombinations, and the various founder alleles that could have been the donor for the recombinations. All such recombinations involve one or more amino-acid substitutions in the α_1 and α_2 domains that alter the interactions of HLA-B with antigenic peptides (*p*) and T-cell receptors (*t*). For each recombination the potential donor alleles are indicated by the coloured boxes on the right and the donated sequences by the coloured residue boxes on the left. For the six recombinations with more than one possible donor, the residues are only shaded with the colour of one of them (the one furthest to the left). b | The ‘heat map’ shows the geographical distribution of the archaic allele HLA-B*73. The colour spectrum denotes increasing frequency from 0% (dark blue) to 4.5% (bright red). Gray shading indicates regions for which high-resolution HLA typing data from ethnically well-defined indigenous populations were not available. The table below the heat map shows the 41 amino-acid differences that distinguish HLA-B*35:01 and HLA-B*73:01. Of these 16 are at functionally important positions that make contact with peptide (*p*), T-cell receptor (*t*), KIR (*k*) or leukocyte immunoglobulin-like receptor (*l*).