Maternal KIR and fetal HLA-C: a fine balance

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ABSTRACT

NK cell effector function is regulated by a range of activating and inhibitory receptors, and many of their known ligands are MHC class I molecules. Human NK receptors encoded by the Killer immunoglobulin-like receptor (*KIR*) gene family recognize polymorphic HLA-C as well as some HLA-A and HLA-B molecules. KIRs are expressed by uterine NK (uNK) cells, which are distinctive NK cells directly in contact with the invading fetal placental cells that transform the uterine arteries during the first trimester. Trophoblast cells express both maternal and paternal HLA-C allotypes and can therefore potentially interact with KIRs expressed by uNK. Therefore, allorecognition of paternal HLA-C by maternal KIR might influence trophoblast invasion and vascular remodeling, with subsequent effects on placental development and the outcome of pregnancy. We discuss here the studies relating to KIR/HLA-C interactions with an emphasis on how these function during pregnancy to regulate placentation. *J. Leukoc. Biol.* **90: 703–716; 2011.**

Introduction

Despite a growing understanding of immune responses and tolerance, pregnancy remains an immunological paradox because maternal immune cells naturally contact the semiallogeneic placenta. These contacts are particularly extensive in species that have evolved invasive hemochorial placentation. In humans, this is characterized by particularly deep placental invasion, which results in transformation of the spiral arteries into low-pressure, high blood flow vessels with concomitant, extensive decidualization of the uterine mucosa [1]. Decidualization is always associated with the presence of a unique and distinct lymphocyte population, the uNK, representing up to

70% of the leukocyte population in first trimester decidua [2]. Because uNK accumulate around infiltrating trophoblast cells, they are obvious candidates for fine-tuning maternal immune responses that might regulate placentation. In contrast, the role of the other uterine immune cell populations (macrophages, dentritic cells, and T cells) may be more important in ensuring immunological tolerance of the maternal adaptive immune system during pregnancy [3–7].

There are two main trophoblast subpopulations: villous trophoblast covers the villous tree and contacts maternal blood in the intervillous space whilst EVT invades the decidua, spiral arteries, and myometrium [1, 8]. Villous trophoblast never express HLA class I or class II molecules, but EVTs have an unusual MHC repertoire with expression of oligomorphic, nonclassical class I HLA-E and HLA-G and the classical polymorphic HLA-C [9–14]. All HLA-C allotypes are ligands for the highly variable KIRs expressed by uNK cells [15–18]. HLA-E, loaded with the leader sequence of other HLA class I molecules, including HLA-G, is also a ligand for most uNK cells because it binds the lectin-like inhibitory receptor CD94/NKG2A, expressed by >90% of uNK cells [19]. HLA-G binds to members of the LILR family; LILRB1 and LILRB2 are found on decidual macrophages and a minority of uNK cells [3, 20]. Some evidence suggests that HLA-G also binds one KIR, KIR2DL4, but whether uNK cells express KIR2DL4 is still controversial [21]. Both *KIR* and *LILR* genes map to the *LRC* on chromosome 19 [22].

Because both *KIR* and *HLA-C* are highly polymorphic, each pregnancy will be characterized by particular combinations of maternal *KIR* and fetal *HLA-C* variants. The maternal *HLA-C* genotype is also important to consider, as it may influence the repertoire and function of uNK cells by interactions of maternal KIR with her self HLA-C molecules during NK cell development, a process called "licensing" or "education" [23, 24]. Failure of placentation where trophoblast invasion is defective and the arteries are incompletely transformed occurs in major disorders of pregnancy, such as pre-eclampsia, FGR, and recurrent miscarriage [1]. In these women, particular combinations

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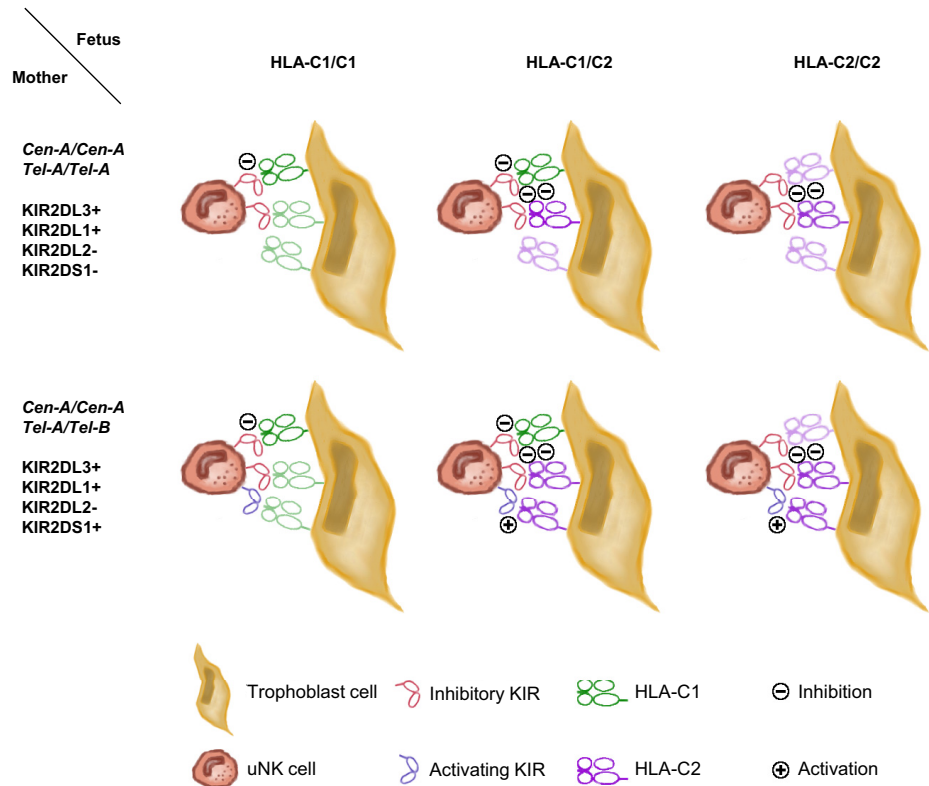
Figure 1. Interactions between maternal KIR and fetal HLA-C. Two common maternal genetic backgrounds are shown: *KIRA/A* (only *Cen-A* and *Tel-A* regions) or *KIRA/Tel-B* (only *Cen-A* plus *Tel-A* and *Tel-B* regions) in combination with three possible fetal *HLA-C* genotypes. For simplicity, the HLA-C status of the mother is considered as C1/C2.

of maternal *KIR* and *HLA-C* variants, together with fetal *HLA-C* groups, are over-represented (**Fig. 1**) [25–27].

Thus, understanding the characteristics of HLA-C molecules, especially when expressed by trophoblast cells, and in particular, their interaction with different maternal KIRs, is crucial to understand how reproductive success may be affected by the maternal immune system. Here, we review current knowledge about HLA-C, HLA-C-binding KIRs, evolution of MHC/KIR interactions, and immunogenetics of pregnancy disorders.

HLA-C

HLA-C molecules, like other HLA class I antigens, are heterotrimers consisting of a 45-kD glycoprotein, an invariant 12-kD β2m, and a bound peptide. The cell surface proteins are highly polymorphic, with 1016 alleles known, encoding 750 proteins (IPD-HLA database, March 2011) [28, 29]. Low expression levels at the cell surface (10% of the levels of HLA-A or -B molecules) are characteristic of HLA-C [30, 31]. This may be a result of unstable mRNA or incomplete protein maturation and may also vary with cell type, a "signature" of those tissues, as well as the infected status of the cell [32]. HLA-C heavy chains associate poorly with β2m, leading to an accumulation of free heavy chain in the ER. When properly assembled, they are not released from the ER because of a stable association with transporters associated with antigen processing (TAP) [33]. In addition, SNP variant (C/T), located −35 kb from the start codon, correlates with expression levels at the cell surface, so that individuals with the C allele of the SNP

have increased levels [34]. This is now shown to be a result of differential binding of microRNA miR-148 to a variant in the 3′-untranslated region [35].

Compared with HLA-A or -B, HLA-C allotypes are more similar to each other, and the peptide-binding domains are more conserved, with a specific KYRV motif at residues 66, 67, 69, and 76 in the α1 helix in keeping with observations suggesting binding of a restricted set of self-peptides [36, 37]. The peptide-binding groove of HLA-C is defined by a glycine at position 45 in the α1 helix and four unique residues in the α2 helix with a reduced diversity compared with HLA-A or -B [36, 38]. HLA-C might be specialized in binding and presenting only specific peptides, possibly derived from viral proteins. That HLA-C has a role in the control of viral infection is suggested from the HIV Nef-mediated down-regulation of only HLA-A and -B and not HLA-C and -E [39]. This can be explained by the absence of one or more of the three critical residues, tyrosine 320, alanine 324, and asparagine 327, in their cytoplasmic tail [40]. Thus, HIV can down-regulate T cell ligands, HLA-A and -B, without triggering NK cell responses to missing self.

Although HLA-C-restricted T cells have been found in transplant recipients and HIV-infected patients, a major role of HLA-C is as a ligand for KIR [41, 42]. Co-crystal structures of the KIR that bind to HLA-C show that a dimorphic residue in position 80 of the HLA-C α1 helix is associated with these KIRs binding with different affinity [43, 44]. Positions 7 and 8 at the C terminus of the peptides also affect KIR recognition, unlike the contact sites of the TCR, which are more central,
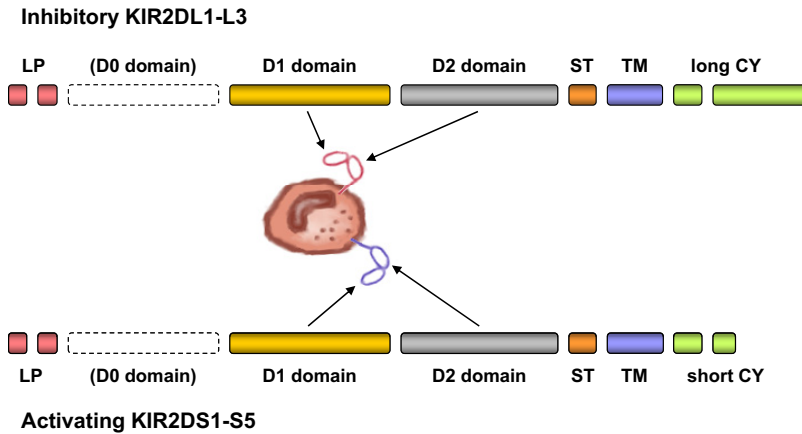
Figure 2. Type 1 KIR2D gene and protein organization. Exons but not introns are shown to scale. LP, Leader peptide domain; ST, stem domain; TM, transmembrane domain; CY, cytoplasmic domain. The pseudoexon is shown as a dotted box.

involving residues 4–6 of the peptide [45, 46]. The footprint of KIR on HLA-C is different from that of TCR with 16 KIR interface residues observed, but KIR and TCR binding areas do overlap such that a HLA-C molecule cannot be bound by a KIR and a TCR simultaneously [47].

A further difference from HLA-A and -B molecules is that HLA-C glycoproteins have different N-linked oligosaccharides at position 86 of the heavy chain. In addition to the two predominant glycan structures found on HLA-A and -B molecules, HLA-C molecules have two other glycans. Those oligosaccharide structures vary with different HLA-C allotypes, but no major consequences on KIR binding have yet been shown [48].

The trophoblast cells invading the maternal uterine tissues express HLA-C at high levels in a stable β2m-associated conformation [13, 27]. On trophoblast cells freshly isolated from normal human pregnancies, HLA-C molecules can only be immunoprecipitated using mAb such as W6/32, which recognize the β2m-associated conformation. In contrast, similar experiments with PBMCs show that unfolded conformers reactive with mAbs to heavy chains and peptide-free class I molecules are also abundant [13]. HLA-C+ trophoblast cells come into direct contact with maternal NK cells expressing KIR at the site of placentation [27].

That KIR/HLA-C interactions are of particular biological importance in the regulation of placentation is suggested by several observations. The KIRs that bind HLA-C are expressed at higher frequency on uNK cells than on pNKs taken from the same woman at the same time. This KIR expression is highest earlier in gestation (6 weeks) and slowly declines over the first trimester [15, 18]. Whether this is a result of expansion of these NK cells that are expressing HLA-C specific KIR is not known, but uNK cells do proliferate in vivo [2, 49]. Binding of KIR-Fc fusion proteins to trophoblast HLA-C molecules and vice versa of HLA-C tetramers to uNK cells shows that direct engagement between maternal lymphocytes and fetal trophoblast can occur [18, 27]. The functions of uNK cells are still somewhat elusive, however, and they are, despite prominent cytoplasmic granules containing perforin and granzymes, poor killers [19, 20, 50, 51]. A range of cytokines and chemokines is produced including VEGF, placenta growth factor, IFN-γ, GM-CSF, IL-8, and MIP-1α [52–55]. Exactly how these studies relate to cytokine production in vivo is unclear

because the protocols used to isolate and measure cytokines vary considerably between different laboratories. In particular, the uNK cells have often been activated by prolonged culture with IL-2 or IL-15 before analysis [53]. Little IFN-γ or IL-8 production is detectable when using freshly isolated uNK cells [20]. Whether uNK cells affect the ability of trophoblast to invade and transform the spiral arteries and/or act directly on the arteries, their most likely function seems to be to regulate blood flow to the intervillous space by regulating trophoblast transformation of arteries [1]. This means that a boundary is formed between the two individuals, the mother and her child, so that normal fetal growth and development can occur without endangering the mother.

KIR

The human *KIR* gene family contains 12 genes and two pseudogenes that are closely linked on chromosome 19q13.4 in the *LRC* [16, 56–58]. KIR are named based on the number of extracellular Ig-like domains (2D or 3D) and by the length of their cytoplasmic tail (L for long and S for short; **Fig. 2**). The 2DL and 3DL KIRs have an ITIM with the consensus sequence Ile/Val/Leu/Ser-x-Tyr-x-x-Leu/Val in the cytoplasmic tail, which is phosphorylated upon binding to a MHC class I ligand [59]. Phosphatases such as SHP-1 are recruited, and the inhibitory cascade follows. Most KIR2DL possess two ITIM motifs in their cytoplasmic tail, but there are exceptions. For example, KIR2DL4 has a single ITIM, in addition to a positively charged arginine in the transmembrane region. Activating KIRs (2DS or 3DS) have short cytoplasmic regions with a charged residue in the transmembrane region and function using adaptor proteins (e.g., DAP-12) containing an ITAM with a consensus sequence Asp/Glu-x-x-Tyr-x-x-Leu/Ile-x$_{(6-8)}$-Tyr-x-x-Leu/Ile [60].

KIRs interact with the top of the α-helices of HLA class I molecules and positions 7 and 8 of the bound peptide with a footprint in the carboxy-terminal region of the α1 helix and the amino-terminal region of the α2 helix [43]. KIR2DL1/2/3 are inhibitory receptors for HLA-C, and binding to HLA-C requires the presence of both D1 and D2 domains of these KIR2D receptors (Fig. 2) [61]. Crystal structures of the KIR2D

TABLE 1. HLA-C Type Ligand Specificities and Frequencies

HLA-C type	Binding affinity					Frequency (%)		
	KIR2DL1	KIR2DS1	KIR2DL2	KIR2DL3	KIR2DS4	Black	Caucasian	Far East Asian
C1								
Cw*01	−	−	++	++	+	1.21	4.02	14.43
Cw*03	−	−	++	++	−	7.83	10.21	25.44
Cw*07	−	−	++	++	−	21.07	27.27	20.22
Cw*08	−	−	++	++	−	4.92	4.19	11.84
Cw*12	−	−	++	++	−	2.03	8.27	5.98
Cw*14	−	−	++	++	+	2.45	2.23	5.62
Cw*15:07	−	−	++	++		0.00	0.00	1.10
Cw*16	−	−	++	++	+	9.87	4.34	1.07
C2								
Cw*02	+++	+	+	+/−	+	9.79	5.11	1.36
Cw*04	+++	+	+/−	+/−	+	17.32	13.10	10.37
Cw*05	+++	+	+	+/−	+	1.98	7.34	0.87
Cw*06	+++	+			−	11.38	9.07	4.50
Cw*07:07, *07:09	+++	+				0.00	0.00	0.00
Cw*12:04, *12:05	+++	+				0.25	1.00	0.00
Cw*15	+++	+			−	1.58	3.66	3.83
Cw*16:02	+++	+				0.58	1.10	0.43
Cw*17	+++	+			−	7.95	1.33	0.34
Cw*18	+++	+			−	3.35	0.48	0.50

Level of binding of five KIRs to HLA-C type and subtype is indicated [64,65]. Blank indicates data not available. Frequencies are average frequencies in all populations recorded in The Allele Frequency Net Database in March 2011 for the three ethnic origins [66]. Some HLA-C subtypes known to differ in their specificity within their "two-digit" type group are specified.

bound to HLA-C allotypes show that although multiple polymorphic residues contribute to the HLA-C binding site on the KIR proteins, a dimorphism at positions 77 and 80 of the HLA-C α1 domain discriminates between two groups of HLA-C allotypes, C1 (Ser[77], Asn[80]) and C2 (Asn[77], Lys[80]) [62, 63] (**Table 1**). KIR2DL1 has specificity for C2 allotypes, and although generally KIR2DL2/L3 have specificity for C1 allotypes, some exceptions have been reported [64]. The comparison between crystal structures of KIR2DL2/HLA-Cw3 (C1) and KIR2DL1/HLA-Cw4 (C2) shows that, out of the 16 interface residues identified, all are conserved, apart from residues 44 and 70. Lys[44] of KIR2DL2 forms a hydrogen bond with Asn[80] of HLA-Cw3, and this cannot occur with KIR2DL1, which has Met[44] instead. The Lys[44] in KIR2DL2/L3 is unfavorable in terms of electrostatic and steric interactions for binding of Lys[80] to C2 allotypes [47]. Thus mutating residues at position 44, Met[44] of KIR2DL2 or Lys[44] of KIR2DL1 is sufficient to switch allotype specificity [67].

In summary, allotypic recognition of C1 or C2 molecules by KIR depends critically on specific interactions between position 44 of KIR and position 80 of HLA-C. Binding measurements indicate that the interaction of C2 with KIR2DL1 is stronger and more specific than that of C1 with either KIR2DL2 or KIR2DL3 [43, 68]. The activating KIR are less well-understood, but KIR2DS1 binding to C2 allotypes has been well-defined [69, 70]. In contrast, KIR2DS2 has never been shown to bind any HLA class I molecules. The only other activating KIR with defined binding specificity is KIR2DS4, which does also bind to some C1 and C2 allotypes, as well as an HLA-A allotype, HLA-A*11 [65, 71].

The *KIR* family is rapidly evolving and is highly variable both in the number of genes that encode activating and inhibitory receptors and in allelic polymorphism at individual *KIR* loci (IPD-HLA database, March 2011) [29]. Based simply on the distribution of activating *KIRs*, two basic *KIR* haplotypes can be distinguished: *A* and *B* (**Fig. 3**). Generally, *A* haplotypes have a smaller number of genes all encoding inhibitory *KIRs* (apart from *KIR2DS4*), whereas *B* haplotypes have additional *KIRs*, most of which are activating. Three framework genes are shared by all haplotypes: *KIR3DL3*, *KIR2DL4*, and *KIR3DL2*. *A* and *B* haplotypes are variable in terms of gene content and combination of genes, especially the *B* haplotype. An individual's *KIR* genotype can be *A/A* (0–1 activating *KIR*), *A/B* (1–6 activating *KIRs*), or *B/B* (3–10 activating *KIRs*). In addition, each *KIR* gene is polymorphic with inhibitory *KIRs* showing more variability than activating *KIRs* [72]. *KIR2DL2* and *KIR2DL3*, as well as *KIR3DL1* and *KIR3DS1*, are now considered as different alleles of the same gene; the sequences have different names, as they were described originally as different genes.

Although many *KIR* haplotypes have been described in different parts of the world, population studies suggest that the majority of *KIR* haplotypes can be classified more simply by consideration of the centromeric and telomeric regions separated by one recombination breakpoint, located between *KIR2DL1* and *KIR2DL4* [72–75]. The centromeric region of the *A* haplotype (*Cen-A*) is characterized by the presence of *KIR2DL1* and *KIR2DL3*, binding C2 and C1, respectively. The centromeric *B* region (*Cen-B*) is defined by *KIR2DS2* and *KIR2DL2*, with *KIR2DL2* binding to C1 and potentially some
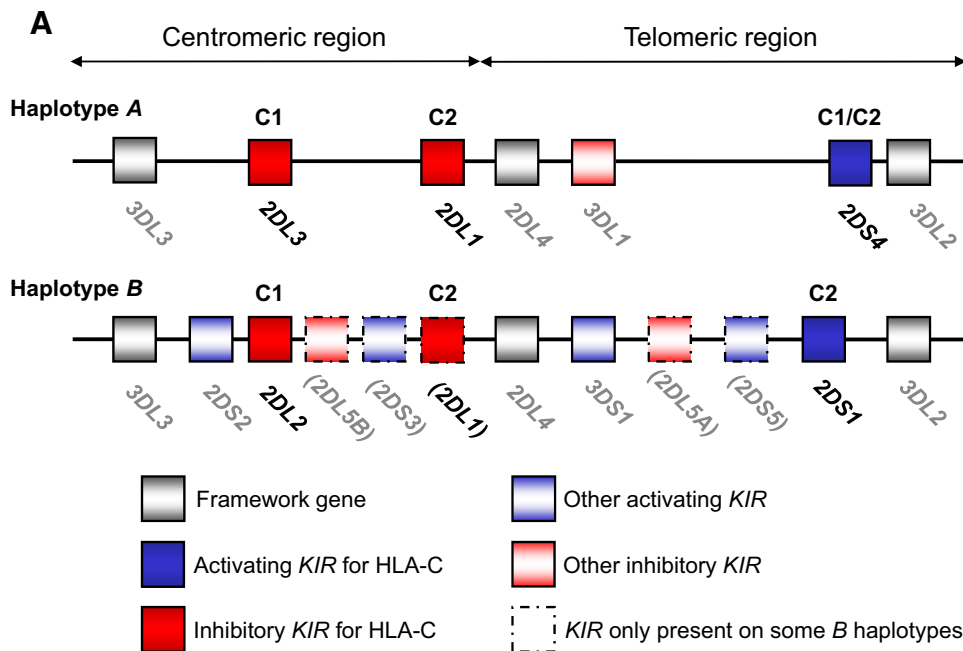
Figure 3. Framework *KIR* and the *KIR*-encoding HLA-C receptors. (A) Representative *KIR A* and *B* haplotypes. Gray boxes = framework *KIR* gene; blue boxes = *KIR* gene encoding an activating receptor for HLA-C; red boxes = *KIR* gene encoding an inhibitory receptor for HLA-C; shaded blue boxes = *KIR* gene encoding activating receptors for other ligands; shaded red boxes = *KIR* gene encoding inhibitory receptors for other ligands; boxes outlined with a dotted line, *KIR* gene only present on some *B* haplotypes. (B) Binding characteristics of the four KIRs for HLA-C2.

Receptor	HLA-C2 ligand	Function	*KIR* region
KIR2DL1	C2	Strong inhibition	*Cen-A*
KIR2DL2	some C2	Weak inhibition	*Cen-B*
KIR2DS4	some C2	Weak activation	*Tel-A*
KIR2DS1	C2	Strong activation	*Tel-B*

C2 allotypes. *KIR3DL1* and *KIR2DS4* are located in the telomeric region (*Tel-A*), and defining the telomeric *B* region (*Tel-B*) are *KIR3DS1* and *KIR2DS1*. Thus, the telomeric regions of both *A* and *B* haplotypes each contain an activating *KIR* for HLA-C molecules: *KIR2DS4* on *Tel-A* binds some C1 and C2 allotypes, whilst *KIR2DS1* on *Tel-B* binds to all C2 allotypes. In contrast, the inhibitory *KIRs* for C1 and C2 are both in the centromeric regions.
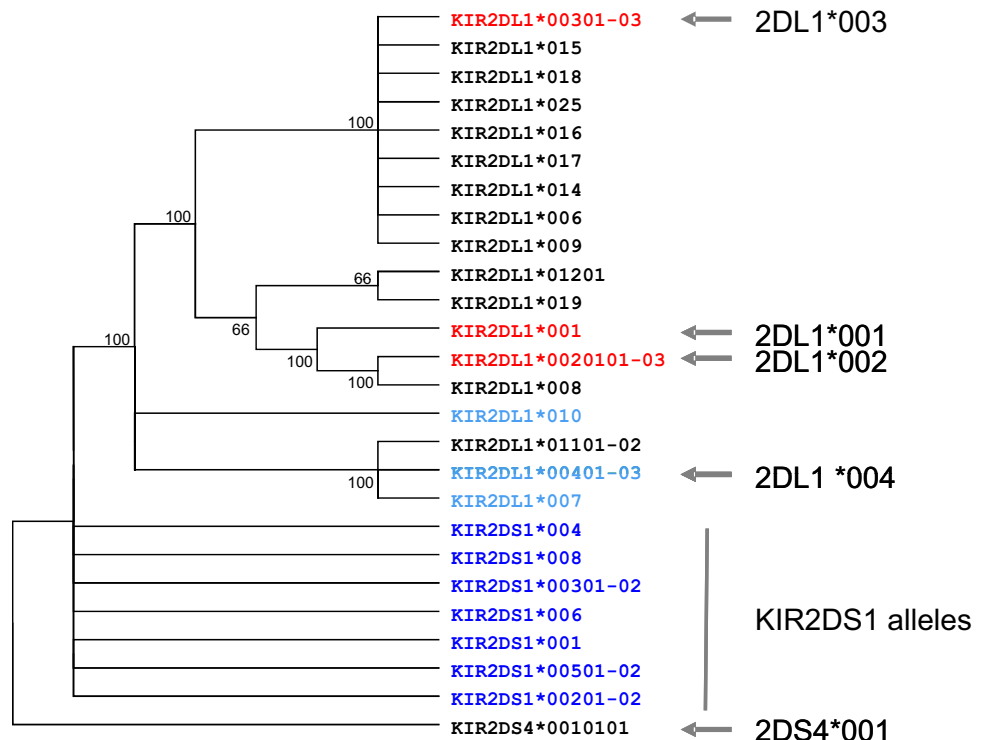
KIR2DL1

A typical *KIR A* haplotype is characterized by the presence of *KIR2DL1* in the *Cen-A* region, so that individuals who have *KIR A/A* genotypes will have two copies of the gene. However, some *Cen-B* haplotypes also have *KIR2DL1*, so that *KIR A/B* or *B/B* individuals may also have two copies of *KIR2DL1* [72]. At present, determining *KIR* copy number is not easy (although several techniques, mostly based on quantitative PCR, have been published [76, 77]). This means that in routine *KIR* genotyping, when only the presence/absence of a *KIR* gene is determined, the number of copies of *KIR2DL1* will not be known. The copy number is likely to influence mRNA transcript level and the proportion of an individual's NK cells that expresses the KIR [77, 78]. It will be of interest in the future to consider *KIR* copy number in population- or disease-association studies.

There are 43 *KIR2DL1* alleles described so far, encoding 25 proteins, with common alleles linked to either *Cen-A* or *Cen-B* haplotypes: *KIR2DL1*001*, *-*002*, and *-*003* are on *Cen-A* and *KIR2DL1*004*, *-*007*, and *-*010* on *Cen-B* [72]. Of note is that the regions encoding D2 domains of the *Cen-B KIR2DL1* alleles are closely related to the *KIR2DS1* gene located on *Tel-B*, making the proteins encoded by the *Cen-B KIR2DL1* alleles significantly different from the *Cen-A KIR2DL1* alleles (**Fig. 4**). Thus, like the allelic *KIR*, *KIR2DL2/L3*, and *KIR3DL1/S1*, particular *KIR2DL1* alleles are characteristic of *A* and *B* haplotypes.

Similar to several studies of *KIR3DL1* variants, different *KIR2DL1* alleles also show variations in expression levels and functional responses. In individuals with one copy of *Cen-A KIR2DL1*00302*, mRNA levels are significantly higher than *Cen-A KIR2DL1*002*. Overall, in *Cen-A* and *Cen-B KIR2DL1* alleles, the trend for levels of mRNA is: *KIR2DL1*00302* > *-*001* > *-*002* > *-*00401* [77]. Within *Cen-B KIR2DL1* alleles, *KIR2DL1*010* seems to induce greater inhibition in NK-like YT-Indy cells than *KIR2DL1*004*, as *KIR2DL1*010*-transfected cells show less lysis of 721.221-Cw6 target cells, weaker degranulation (as assessed by CD107 surface expression), and lower IFN-γ production [79]. KIR2DL1 proteins bind with strong affinity to C2 molecules, although it is still unknown how the differ-

Figure 4. Boostrap consensus tree for 18 KIR2DL1 protein variants, seven KIR2DS1 protein variants, and one common KIR2DS4 protein variant. The percentages indicated are based on 1000 boostrap replicates. This tree has been drawn from full-length protein sequences. KIR2DL1 proteins encoded by alleles known to be linked to the *KIR Cen-A* region are in red; KIR2DL1 proteins encoded by alleles known to be linked to the *KIR Cen-B* region are in pale blue; KIR2DS1 proteins are in dark blue.

ent KIR2DL1 alleles bind to a range of C2 allotypes. The functional implications of this variability of both KIR and C2 (and its bound peptide) in infections, tumors, and pregnancy will be important to determine [64, 78].

KIR2DS1

KIR2DS1, like KIR2DL1, binds to C2 allotypes. The extracellular domains of KIR2DS1 are very similar to their inhibitory KIR2DL1 counterparts, but as a result of the different cytoplasmic tail, an activating signal is transmitted. This means that the original mAbs used to detect KIR2DL1 almost invariably also bound KIR2DS1. Recently, a new mAb (clone 143211) has been generated that can discriminate between KIR2DL1 and KIR2DS1, so expression on NK subsets in normal and diseased individuals can now be examined [80–82]. As more mAbs are described, the specificity of individual KIR alleles also will need redefining, as seen for KIR2DL3*005, a KIR2DL3 allele that reacts with mAbs used to detect KIR2DL1/S1, such as EB6 and 11PB6 [83]. That KIR2DS1 can function in vivo is shown in patients receiving HSCT, where proliferation, cytokine production, and cytolysis by KIR2DS1-positive NK cells binding to C2-positive targets have been found [84–86]. When an NK cell expresses both KIR2DL1 and KIR2DS1, the inhibitory signal dominates [81].

KIR2DS1 is located on the *Tel-B* region of *B* haplotypes next to the framework gene *KIR3DL2*. Unlike *KIR2DL1*, which is present in 72–100% in individuals throughout the world, the carrier frequency of *KIR2DS1* is lower, ranging from 13% to 63%, but between 33% and 49% for most populations (when considering only data obtained from a sample size >100; The Allele Frequency Net Database, March

2011, allelefrequencies.net) [66]. This allows informative analysis by genetic epidemiological studies, and several do show an association of *KIR2DS1* at higher frequency than controls for diseases such as psoriasis and autoimmune diseases [87–91]. In Ebola and human papilloma virus infection, a differential outcome is also associated with a higher frequency of *KIR2DS1* [92, 93]. Thus, there is strong potential for KIR2DS1-mediated modulation of NK cell function, as its expression, specificity, and function in disease and allogeneic situations all suggest that this activating KIR, specific for C2 allo plays a key role.

KIR2DL2/L3

C1 allotypes are bound by KIR2DL2 or KIR2DL3, which function as alleles sharing a locus, with *KIR2DL3* located on *Cen-A* and *KIR2DL2* on *Cen-B*. There are >20 alleles at the *KIR2DL2/3* locus. but the common ones are *KIR2DL3*001* (*Cen-A*) and *KIR2DL2*001* or -*003* (*Cen-B*; IPD-HLA database, March 2011) [29, 66, 72]. The binding to C1 allotypes is of lower affinity than that of KIR2DL1 with C2 (see above). In strong LD with *KIR2DL2* is the potential activating *KIR* for C1 allotypes, *KIR2DS2*, but despite their similarity in the extracellular domains, no binding of KIR2DS2 to HLA molecules has been demonstrated. Of particular interest is that binding specificities of KIR2DL3 are mainly to C1 allotypes, whereas KIR2DL2 is more promiscuous and also binds some C2 allotypes (Table 1). The gene *KIR2DL2/L3* is present in all individuals, and *C1* is generally more frequent than *C2*. This means that these weak interactions will be occurring in the majority of individuals.

KIR2DS4

KIR2DS4 is an activating *KIR* located on *Tel-A* with a typical lysine in the transmembrane region and a short cytoplasmic tail [56, 94]. Two main forms are found: most of the *KIR2DS4* alleles so far described have a 22-bp frame-shift deletion in exon 5, which encodes the second extracellular Ig-like domain (D2). This results in a KIR protein with only D1 and without the transmembrane and cytoplasmic domains [73]. Potentially, this deleted form might result in expression of a soluble form of KIR2DS4, but no binding has been demonstrated using Fc-fusion proteins, and low levels of transcripts in NK cells make it unlikely that this KIR variant is functional [65, 73, 77, 95, 96]. Several alleles of both the full-length and the deleted forms exist [97–101].

In contrast, the full-length form of KIR2DS4 is expressed and functional. Although attempts to demonstrate binding to HLA class I molecules were unsuccessful initially, it has now emerged that specificity does not depend on position 80 of HLA-C, so that KIR2DS4 can bind both C1 and C2 allotypes [65, 71]. The C2 allotypes are the same as those bound by KIR2DL2 and are all at significant frequency levels in Black, Caucasian, and Far East Asian populations (Table 1). Cocrystal structure of KIR2DS4 bound to HLA-C shows that the folding topology resembles other KIR2D. KIR2DS4 has a lysine in position 44, similar to KIR2DL2, and this explains its specificity for C1 [65]. However, this Lys^{44} should prevent binding to C2 (see above). It seems that differences in the backbone in KIR2DS4, compared with KIR2DL2/3, increase the distance between this Lys^{44} and residue 80 of HLA-C, explaining the weak affinity for C1. Thus, despite Lys^{44}, interactions of KIR2DS4 with C2 are allowed [65]. As with other KIR, the nature of the bound peptide is probably also important [65].

KIR2DS4 is considered an ancestral *KIR* that arose from a gene-conversion event with *KIR3DL2*, a KIR exquisitely sensitive to particular HLA-A allotypes and their bound peptides [65, 102]. KIR2DS4 is functional, as shown using transfectants and primary NK cells [65]. In genetic studies, associations with malaria, leukemia, and acute graft-versus-host disease (GvHD) are described [103–106]. All these recent findings indicate that KIR2DS4 needs to be considered when KIRs on NK cells interact with HLA-C molecules in disease and pregnancy.

EVOLUTION

MHC-C orthologs are only found in great apes—gorillas, chimpanzees, and orangutans. *C2* alleles are not found in orangutans, and only ~50% of individuals have an *MHC-C1* [102, 107, 108]. In keeping with this, *KIR2D* homologues have been described in orangutan, several with Lys^{44}, which correlates with KIR specificity for C1 in humans, but none have Met^{44}, the residue implicated in C2 binding. In addition, several orangutan KIR2D have Glu^{44}, and these KIR have arisen more recently than Lys^{44} [109]. Thus, the orangutan resembles an evolutionary intermediate, in which C1 epitopes and C1-specific *KIRs* are in place, but the C2 epitope and C2-specific *KIRs* have not yet appeared. The expansion of *KIR2D* in orangutan has occurred in the same time-frame as the emergence of

MHC-C, showing how strongly interactions between KIR and MHC-C influence their evolution. The stronger interaction between MHC-C2 and KIR, therefore, arose later in the great apes and became fixed, so that now both *C1* and *C2* alleles are represented in all human populations.

KIR genes have been analyzed extensively in chimpanzees, and whereas they have orthologs of all human *HLA class I* genes, chimpanzees have a different organization of *KIR* haplotypes [110]. Similar to humans, chimpanzees have framework genes separating centromeric and telomeric regions, but all of the variable *KIR* genes are restricted to the centromeric part. Thus, as humans evolved, more *KIR* genes moved to the telomeric region, and recombination between the centromeric and telomeric regions became a diversifying mechanism [111]. Knowledge of the organization of *KIR* genes in chimpanzees and orangutans has led to the idea that the *Cen-A/Cen-B* dichotomy pre-existed the *Tel-A/Tel-B* dichotomy, and all four regions existed before the emergence of the modern human species [72]. All present-day human groups have both *KIR A* and *KIR B* haplotypes, indicating that they are essential for survival of human populations [112]. In addition, human-activating KIRs have lower affinity for HLA ligands than those in chimpanzees, so that only human KIR2DS1 and KIR2DS4 still have demonstrable binding to HLA-C allotypes, and this is at much lower affinity than KIR2DL1. Gorillas also have a high-avidity, C1-specific KIR (*Gg-KIR2Dsa*), so disabling the activating, C1-specific KIRs seems to be a uniquely human phenomenon [113].

KIR education and repertoire

The functional KIR repertoire of an individual depends on the *KIR* genotype, and each mature NK cell expresses a specific set of KIRs, a combination of zero to all *KIR* present. The "Product rule" (the probability of two KIR expressed together on an NK cell equals the product of the frequencies of these two KIRs in the NK cell pool) was proposed initially to explain the KIR repertoire [114]. However, the presence of the HLA class I ligand also influences the KIR repertoire, so that mature NK cells tend to express at least one inhibitory receptor for self HLA class I. This effect is particularly observed when the NK cell lacks expression of the alternative inhibitory receptor CD94/NKG2A [115]. More recent findings now suggest that coexpression of multiple self or non-self KIR at the NK cell surface follows a sequential model known as a ligand-instructed model of NK cell receptor acquisition [116, 117]. This is seen particularly in individuals of the *KIR A/A* genotype: they have more NK cells expressing a single KIR corresponding to the individual's HLA-C group: KIR2DL3 for C1 individuals and KIR2DL1 for C2 [117].

The strength of the interactions occurring between inhibitory KIRs and their HLA class I ligands during the development of NK cells determines the education process, leading to both tolerance of self and to the functional responsiveness of NK cells upon encounter with ligands. Thus, if high-affinity engagement of KIR2DL1 with C2 occurs during NK cell development, this strong inhibitory signal results in highly responsive NK cells when the C2 is altered in pathological states. This potentially means that individuals who only have C1 alleles will not have such strong NK responses as those who have C2, be-

cause of the lower-affinity interaction between C1 and KIR. KIR2DS1 functional responses can also be affected by the presence/absence of the C2 ligand, with KIR2DS1+ NK cells "tuned down" in a *C2/C2* donor compared with a *C1/C1* donor [82]. This can over-ride CD94/NKG2A and other inhibitory KIR signals, such as KIR2DL3, but not KIR2DL1. In these experiments with KIR2DS1, the tuning of responsiveness was restricted to target cell recognition, and not from stimulation with exogenous cytokines. These recent findings do suggest that the balance between activating KIR2DS1 and inhibitory KIR2DL1 is critical and is modified by the individual's C1 or C2 status.

All of those experiments have been done using pNK cells, and it is not clear if education will also occur in uNK cells. However, activating and inhibitory KIRs specific for HLA-C, are expressed at higher levels and on an increased proportion of NK cells in the human decidua compared with blood [18]. Furthermore, immature NK cell precursors are present in the uterine mucosa which differentiate into mature NK cells that do express KIRs [118]. uNK cells do therefore exhibit unique KIR profiles, but it cannot be assumed that they follow the education/licensing rules defined for pNK cells. Indeed, it seems unlikely to be the same because >90% of uNK cells express the alternative inhibitory NKR, CD94/NKG2A, at high levels, which is also capable of educating NK cells [19]. Furthermore, they show features of activation, such as CD69 expression, and activated NK cells can over-ride education via KIRs [115, 119, 120].

KIR summary

By comparing and contrasting *A* and *B KIR* haplotypes, it appears that in each *Cen* and *Tel* region, there are genes encoding KIR that can bind different subsets of HLA-C allotypes with different affinities (Fig. 3). This means that in any individual, the strength of systemic NK responses will depend on: i) which alleles of inhibitory *KIR2DL1/2/3* and activating *KIR2DS1/S4* are present and ii) which *C1* and *C2* alleles an individual inherits. These interactions during NK development will set the threshold for NK responsiveness when HLA-C molecules are altered during disease as a result of either downregulation or of peptide modifications. An important proviso is that it is still unknown whether NK education is similar in the uterine microenvironment. In the context of pregnancy though, the paternal *HLA-C* allele inherited by the fetus is a third variable likely to be of critical importance. Whether the paternal HLA-C additionally impacts on uNK cell education or is the target of allorecognition during uNK cells effector responses is also unknown.

DISEASE ASSOCIATIONS

A summary of genetic epidemiological studies of *KIR* and *HLA-C* associations is shown in **Table 2**. We have omitted studies that have only assessed *KIR* variability without analysis of the *HLA-C* genotype. The most robust study relating to response to infection is still that for HCV, where individuals homozygous for *KIR2DL3* (i.e., *Cen-A/Cen-A*) and *C1* alleles re-

spond better to HCV, and certain *C1* alleles seem to be particularly protective [121–123].

Many of the other studies have small numbers of patients and controls, and other problems make comparisons difficult. For example, frequently, particular *KIR* genes are highlighted without taking into account the strong LD in the centromeric and telomeric regions of *KIR A* and *B* haplotypes [145]. Thus, selecting certain genes such as *KIR2DS2* or *KIR2DS5* may be misleading as they are in almost perfect LD with major KIRs with known ligands (*KIR2DL2* or *KIR2DS1*). The definition of the common *Cen-A*, *Cen-B*, *Tel-A*, and *Tel-B* regions may facilitate more conformity in these studies (see Table 2), but without the raw data it has been difficult to assign all genotypes accurately. By analyzing the main *KIR* regions in clinical situations where HLA-C is likely to play a role, the *KIR* associated with particular disorders may be inferred. The difficulty in analyzing copy number variation in disease association studies also means that important KIRs such as *KIR2DL1* are not considered informative in terms of presence/absence, as it is present in the vast majority of individuals. A further consideration is the wide variation seen in different populations, so that disease association studies across the world cannot be compared easily [146].

At present, it is difficult to draw meaningful conclusions or any simple message. There is speculation that *KIR A* haplotypes are beneficial in response to infectious disease, whereas *KIR B* haplotypes associate with autoimmune conditions, but this will need larger and more standardized analyses of *KIR* and *HLA class I* interactions in association studies similar to those done for HIV and HCV [121, 147, 148]. This would be in keeping with *KIR A/A* individuals theoretically having more responsive NK cells when self-HLA is perturbed in disease because of the strong inhibitory signals received during NK development. It is also uncertain from the findings to date why *C2* alleles have been selected and become fixed in great apes. Despite the negative effect of C2 when present in the fetus in pregnancy, there is no obvious protective effect in infectious disease association studies. Of interest though is that when there is a fetal *C2* allele present, mothers who lack *C2* (i.e., *C1/C1*) are more at risk than *C2/x* mothers, so *C2* may be protective for mothers but a problem if present in the fetus [27].

All of these problems are exemplified by the reported studies about recurrent miscarriage, where different associations of *KIR/HLA-C* genotypes have been detected. All are small case control studies with no consensus on clinical criteria and appropriate choice of controls, copy number has not been defined, the analysis has been carried out quite differently in each study, and two are from the United Kingdom, one from India, and one from China [26, 27, 143, 144].

KIR AND HLA-C IN ALLOGENEIC SITUATIONS: HSCT AND PREGNANCY

A clinical scenario where KIR/HLA-C interactions are important is in HSCT, where donor cells are grafted into bone marrow-ablated recipients. When comparing the studies that have looked at *KIRs* in HSCT, all have different patient characteristics, especially in terms of T cell depletion in the graft, and

TABLE 2. Summary of *KIR/HLA-C* Disease Associations Revealing Direct Interactions

Disease	*KIR* genes	*KIR* region	*HLA-C* group	Effect	Patients	Controls	Reference
Infectious diseases							
HCV	*KIR2DL3*	*Cen-A*	*C1*	Resolution of infection	685	352	[121]
Chronic hepatitis C infection	*KIR2DL3* homozygosity	*Cen-A/Cen-A*	*C1/C1*	Response to treatment	77	109	[122]
Chronic hepatitis C infection	*KIR2DS3*	*Cen-B*	*C2*	Susceptibility	296	247	[123]
HCV	*KIR2DL2/L3* + *KIR2DS4*	*Tel-A*	*C1*	Protection	160	92	[124]
Chronic HBV	*KIR2DL1*	*Cen-A*	*C2*	Susceptibility	182	140	[125]
Vertical HIV-1 transmission	*KIR2DL3* homozygosity	*Cen-A/Cen-A*	*C1/C2*	Susceptibility	28	150	[126]
Autoimmune and inflammatory conditions							
Acute kidney graft rejection	*KIR2DS5*	*Tel-B*	*C1*	Protection	280	690	[127]
Ankylosing spondylitis	*KIR2DS1*	*Tel-B*	*C2*	Susceptibility	115	119	[128]
Ankylosing spondylitis	*KIR2DS5*	*Tel-B*	*C1*	Protection	101	690	[127]
Crohn's disease	*KIR2DL2*	*Cen-B*	*C2*	Protection	1,306	299	[129]
Crohn's disease and ulcerative colitis	*KIR2DL1* or *KIR2DL1* and their respective ligand	*Cen-A* or *Cen-B*	*C2* or *C1*	Protection	248	250	[130]
Type 1 diabetes	*KIR2DS2*	*Cen-B*	*C1*	Susceptibility	149	207	[131]
Idiopathic bronchiectasis	*KIR2DS1* and *KIR2DS2*	*Cen-B, Tel-B*	*C1*	Susceptibility	96	101	[132]
Multiple sclerosis	*KIR2DS1*	*Tel-B*	*C2*	Protection	121	103	[91]
Primary sclerosing cholangitis	*KIR2DL1* (and *KIR3DL1*)	*Cen-A*	*C2*	Protection	365	368	[133]
Psoriasis	*KIR2DS1*	*Tel-B*	*C2*	Susceptibility	116	123	[134]
Psoriatic arthritis	*KIR2DS2* or *-S1* and, respectively missing *KIR2DL2/3* or *-L1* and HLA-C ligand	*Cen-B* or *Tel-B*	*C2* or *C1*	Susceptibility	366	299	[87]
Rheumatoid arthritis	*KIR2DS2/L2*	*Cen-B*	*C1/C2*	Response to therapy	66	100	[135]
Rheumatoid arthritis	Absence of *KIR2DS5*	*Tel-A*	*C2*	Susceptibility	366	690	[127]
Rheumatoid vasculitis	*KIR2DS2*	*Cen-B*	*C1*	Susceptibility	70+30	76	[136]
Type 1 diabetes	*KIR2DL1* present, *KIR2DL2* absent	*Cen-A/Cen-A*	*C2*	Protection	248	250	[137]
Cancer							
Cervical cancer	*KIR3DS1*	*Tel-B*	*C1*	Susceptibility	196	330	[138]
Malignant melanoma	*KIR2DL3*	*Cen-A*	*C1*	Susceptibility	50	54	[139]
Chronic myeloid leukemia	*KIRDLS2/L2*	*Cen-B*	*C1*	Protection	52	154	[140]
HSCT							
Hematologic malignancy	Absence of *KIR2DS1* in donor	*Tel-A*	*C2*	Survival	59		[85]
Myeloid leukemia	*KIR2DS2* in donor	*Cen-B*	*C2*	Susceptibility	220		[141]
Myeloid leukemia	Any *B* haplotype *KIR* in donor	*Cen-B* or *Tel-B* (*B/x*)	Matched *C*	Survival	448		[142]
Reproduction							
Pre-eclampsia	Absence of *B* haplotype *KIR*	*A/A*	Fetal *C2*	Susceptibility	200	201	[25]
Pre-eclampsia, FGR, recurrent miscarriage	Absence of *B* haplotype *KIR*	*A/A*	Fetal *C2*	Susceptibility	941	592	[26]
Recurrent miscarriage	Absence of *KIR2DS1*	*A/A*	*C2*	Susceptibility	269	95	[27]
Recurrent miscarriage	*KIR2DS2* and absence of *KIR2DL1*	*Cen-B*	*C1*	Susceptibility	177	200	[143]
Recurrent spontaneous abortion	*KIR2DS2*	*Cen-B*	*C1*	Susceptibility	73	68	[144]

this again leads to difficulty in interpretation of results (Table 2). Outcomes in terms of both GvL and relapse or survival were assessed. A GvL effect, as a result of donor NK cells killing recipient leukemic cells, has been found in T cell-depleted HSCT, which depends on the donor *KIR* and recipient *HLA* genotype, although the most important criterion for donor selection remains *HLA class I* and *class II* matching [149]. As the *KIR* and *HLA* genes are on separate chromosomes, different donor/recipient *HLA* and *KIR* combinations can be found, and several cohorts have been studied for outcomes. In the biggest study, the presence of the *Cen-B* or *Tel-B* regions in the donor is beneficial in terms of protection from relapse and survival in patients with AML but not ALL [75]. Two *KIR B* regions correlated with better outcomes than one *B* region, and within the donors with two *B* regions, two *Cen-B* regions were associated with better outcomes.

In genetic epidemiological studies of pregnancy, consideration not only of maternal *KIR* and *HLA-C*, but also the fetal *C* group is necessary. This is analogous to HSCT, where donor *KIR*, donor *HLA-C*, and recipient *HLA-C* may all be important. That KIR/HLA-C interactions are likely to play an important role in regulating placentation has come from studies of normal pregnancies compared with those where trophoblast invasion is defective. Clinically, failure of placentation can present as pre-eclampsia, FGR, or recurrent miscarriage, and these disorders represent a spectrum reflecting the degree to which the trophoblast invasion is affected. Indeed, in all pregnancies with these clinical problems compared with normal control pregnancies, the maternal *KIR A/A* genotype is increased in frequency in combination with a fetus carrying a *C2* group [25–27]. More recent analysis confirms that the *KIR B* haplotype provides protection, particularly those *KIRs* located in the *Tel-B* region. Importantly, this is where *KIR2DS1*, encoding the activating KIR for C2 allotypes, is found. Although still preliminary, women with a *KIR A/A* genotype with a *C2* in the fetus seem most at risk if the fetal *C2* is derived from the father, and they themselves lack *C2* (i.e., *C1/C1* homozygous mothers) [27]. These findings point to a crucial role of paternal MHC in maternal-fetal interactions during placentation. This is also suggested from mouse models where paternal MHC class I expression has been detected at the surface of the invasive trophoblast giant cells. Certain mating combinations of mouse strains differing in their *MHC* haplotype influence uterine vasculature and, consequently, placental and fetal growth [150].

It will be critical in future studies to type alleles of both *HLA-C* and of particular *KIRs* such as *KIR2DL1*. Other genes such as *KIR2DS4* (*Tel-A*) or *KIR2DL2* (*Cen-B*), which can bind some *C2* allotypes, should also not be ignored. To date, these findings have only been performed in case-control studies of UK Caucasian women and their offspring. Prospective studies of well-characterized pregnancies, where Doppler ultrasound measurements of uterine artery blood flow are available, are now necessary so that a more direct correlation between *KIR* and *HLA-C* genotypes and uterine artery blood flow can be made. In addition, pregnancies in other ethnic groups should be studied, as *KIR* genotypes vary enormously across different populations. Africans, in particular, will be an important group

to study, as pre-eclampsia is more common in sub-Saharan Africa [151, 152]. Nonetheless, these initial studies do indicate that innate immune system genes are important, not only in determining responses to infection, but also in defining how deeply the placenta taps into the mother's nutrient supply.

It is certainly important to continue to learn from the parallels between cell transplantation and reproduction, where allorecognition by NK cells seems to be a key determinant of the outcome, and *KIR B* haplotypes have a beneficial effect. Whether the two biological situations share similar underlying mechanisms remains to be elucidated.

Humans are under unique selective pressures for reproduction compared with other great apes. This is a result of the constraints imposed by bipedalism on pelvic size and shape, as well as the enormous increase in brain size [153]. Amongst clinicians, this obstetric dilemma is well-known and means that keeping birth weight between the two extremes of FGR (with the associated pre-eclampsia) and obstructed labor from cephalo-pelvic disproportion is a paradigm of stabilizing or balancing selection [154]. In keeping with this, our unpublished results suggest that women who have very large babies (>95th percentile) have an increased frequency of *Tel-B* genes compared with controls [155]. Unraveling the complexity of this KIR/HLA-C immune system and its contrasting roles in immune defense and reproductive success continues to be a major challenge.

AUTHORSHIP

O.C. and S.X. wrote the review. A.M. coordinated, contributed to, and revised the writing and performed most of the research reported.

ACKNOWLEDGMENTS

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KEY WORDS:

innate immunity · reproduction · NK cells · trophoblast · KIR2D · MHC

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Maternal KIR and fetal HLA-C: a fine balance

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