Human leukocyte antigen-C and killer immunoglobulin-like receptors in reproductive medicine

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Abstract

Proper communication between natural killer cells and the human leukocyte antigens of the embryonic trophoblast at the maternal-fetal interface during pregnancy is essential for successful reproduction. However, specific combinations of embryonic human leukocyte antigen-C with killer immunoglobulin-like receptors on decidual natural killer cells (the immunological code of pregnancy) can be associated with obstetric morbidity and pregnancy loss. This article presents an updated review of the mechanisms underlying the interaction between embryonic human leukocyte antigen-C and maternal killer immunoglobulin-like receptors and their relevance to the physiology and pathophysiology of human reproduction.

Main messages

- Pregnancy-related complications represent a real challenge for health professionals, are relatively frequent, and have devastating consequences for patients and their families.
- It is necessary to reveal the mechanisms involving implantation and placental development to improve perinatal outcomes and assisted reproductive therapies.
- Adequate cellular and molecular communication at the maternal-fetal interface are crucial for successful reproduction.
- Certain combinations of embryonic human leukocyte antigens-C present on the trophoblast and immunoglobulin-like receptors on natural killer cells may be associated with obstetric morbidity and gestational loss.

Introduction

Pregnancy-related complications represent a real challenge for health professionals, are relatively frequent, and have devastating consequences for patients and their families [1,2]. Pre-eclampsia affects between 3 and 5% of pregnancies, and it is one of the leading causes of maternal and neonatal morbidity and mortality. Between 15 and 20% of pregnancies end in miscarriage, and up to 5% of couples repeat miscarriages [3]. Although embryonic genetic alterations are considered responsible for most sporadic miscarriages, approximately 40% of euploid embryos used in assisted reproduction either fail to implant or are lost early in pregnancy [4].

Because these diseases have shared pathophysiology, it is essential to reveal the mechanisms involving implantation and placental development to improve perinatal outcomes and assisted reproductive therapies. Although gestation is a multifactorial process, it is currently known that the interaction between embryonic human leukocyte antigens (HLA) and the maternal immune system cells present at the maternal-fetal interface is essential for a successful pregnancy.

The risk of developing pre-eclampsia or eclampsia [5], repeated miscarriages [6], fetal growth disturbances [7,8] and implantation failures in assisted reproductive therapies [9,10] have been related to embryonic HLA-C and the killer immunoglobulin-like receptors (KIR) genotype in the mother's natural killer cells. Today, embryonic HLA-C is considered a risk factor for the development of some of these pathologies.

The present review outlines the main principles of the interaction between embryonic HLA-C and KIR receptors of maternal natural killer cells and the effect on the activity of these cells at the maternal-fetal interface. Moreover, it highlights the importance this process has on placental development, the evolution of pregnancy, and the possible obstetric pathologies that variations in this HLA-C/KIR interaction can explain.

Uterine natural killer cells and the extravillous trophoblast as promoters of placental development

Innate immunity plays a fundamental role in human reproduction. In the decidua, 90% of the maternal immune system cells belong to the innate immunity (uterine natural killer cells and macrophages). Meanwhile, between 3 and 10% correspond to cells of the adaptive immunity, represented mainly by regulatory T lymphocytes [11].

Under basal conditions, the population of uterine natural killer cells in the endometrium varies according to the menstrual cycle: they undergo massive apoptosis in the premenstrual phase, shed with the rest of the endometrium during menstruation [12], and increase progressively after ovulation, especially during the window of implantation. These changes prepare the endometrium for gestation. If gestation occurs, they proliferate even more from early pregnancy and throughout the first trimester, initially concentrating in the basal decidua near the site where implantation occurs [12]. These cells decrease from the second trimester until they return to basal values at the end of pregnancy [13].

In the first trimester of pregnancy, uterine natural killer cells become the majority population and represent approximately 70% of the innate immunity cells [11]. The remaining 20% is composed mainly of macrophages (MΦ), and although these are the most numerous population after uterine natural killer cells, little is known about their role in pregnancy. However, it is known that uterine natural killer cells and macrophages – predominantly of anti-inflammatory or M2 phenotype – participate in a coordinated spiral artery remodeling and production of interleukin (IL)-10 and M-CSF [14]. Also, there is *in vitro* evidence that they can inhibit the activity of T lymphocytes and induce their differentiation into regulatory T cells [15,16].

Although still a matter of debate, it has been shown that uterine natural killer cells have a diverse origin. The unique distribution around embryonic tissues is due to different mechanisms [17]. Uterine natural killer cell progenitors from the bone marrow can proliferate and differentiate into resident natural killer cells under the influence of interleukin-15. This cytokine is produced by endometrial stromal cells in response to the increase in progesterone that occurs after ovulation and during early pregnancy [18]. On the other hand, there is also evidence that endometrial cells and the trophoblast produce chemokines such as CXCL9/Mig, CXCL10/IP-10, CXCL12/SDF-1, CCL4/MIP-1β, and CCL3/MIP-1α. These molecules exert potent chemotactic effects on peripheral blood natural killer cells [19,20,21], favoring their recruitment and migration. Studying the expression of the surface molecules of natural killer cells has allowed us to identify multiple subpopulations (immunophenotypes). These subgroups have different roles within peripheral blood, secondary lymphoid organs, and tissues – such as the endometrium and decidua – where these cells ultimately exert their function.

Classically, natural killer cells have been divided into two major groups according to the density of expression of the cell adhesion molecule CD56 and the presence or absence of CD16 (type III receptor of the low-affinity Fc region of the immunoglobulin G). In peripheral blood, 90-95% of natural killer cells are $CD56^{dim}/CD16^{+}$ type which has a significant cytotoxic capacity and releases interferon-γ rapidly upon activation. $CD56^{bright}/CD16^{-}$ constitutes the remaining 10% of natural killer cells and has less cytotoxic capacity. Instead, the latter type mainly produces cytokines and chemokines, including interferon-γ, tumor necrosis factor-α, interleukin-12, interleukin-15, and granulocyte and monocyte colony-stimulating factor [22]. These natural killer cells are predominant in secondary lymphoid organs and many tissues, including the endometrium and decidua.

Although decidua natural killer cells are mostly $CD56^{bright}/CD16^{-}$, they differentiate from all natural killer cells [23] because they additionally express several molecules (e.g., CD9, CD69, CD49a) that are considered markers of residence [22] and have a unique repertoire of

KIRs [24]. The expression of these receptors is of vital importance at the maternal-fetal interface because the interaction of decidual natural killer cells with a set of ligands selectively expressed by the extravillous trophoblast is favored. These ligands include HLA-C, HLA-E, and HLA-G molecules [25]. This reciprocal interaction capacity has led to the consideration of decidual natural killer cells as regulators of placental development.

Functionally, decidual natural killer cells possess peculiar characteristics. These cells produce vascular endothelial growth factor A and C and placental growth factor, regulating angiogenesis during placental formation [25]. In addition, they release cytokines such as interleukin-8, which promotes cytotrophoblast migration [25], and tumor necrosis factor-α and interferon-γ that inhibit it instead [26]. Moreover, they are an essential source of GM-CSF, CSF-1, and leukemic inhibitory factors that regulate implantation [27].

Under normal conditions, uterine natural killer cells are not directly cytotoxic against the embryo or trophoblast. However, it is important to note that the expression of several activating receptors such as NKp30, NKp44, NKp46, CD244 [13] – and the presence of perforin, granzyme, and granulysin granules – evidence that the cytolytic machinery is intact [28]. In specific situations (e.g., fetal-neonatal autoimmune thrombocytopenia), activation of peripheral blood natural killer cells and antibody-mediated cytotoxicity could play a fundamental role over pathophysiological processes. In animal models, it has been shown that the trophoblast of females sensitized by previous pregnancies expresses platelet antigens that form immunocomplexes capable of activating peripheral blood natural killer cells. These processes cause abortion, chronic villitis, and placental dysfunction that can be reversed with intravenous gamma globulin [29-34].

During gestation, the spiral arteries of the uterus are transformed into flaccid ducts, allowing maternal blood to flow slowly and steadily in the intervillous space to boost the exchange of nutrients and oxygen needed for fetal development [35]. Decidual natural killer cells are in direct contact with the extravillous trophoblast and around the spiral arterioles in early pregnancy [36], regulating trophoblast invasion [37] and remodeling these vessels.

Defective remodeling of the spiral arteries has been associated with various obstetric pathologies: late miscarriage [38], pre-eclampsia, intrauterine growth restriction [39,40], and even preterm delivery [41]. These pathologies are thought to have a common origin in trophoblastic dysfunction and placental development [42]. Moreover, the magnitude of the defect directly influences the obstetric outcome: more severe alterations manifest earlier and cause miscarriage, while milder alterations manifest later and cause intrauterine growth restriction and pre-eclampsia [43].

To remodel the spiral arteries, the villous cytotrophoblast (which is in contact with the decidua at the base of the anchoring villi) separates and forms the extravillous trophoblast. The extravillous trophoblast invades the decidua, reaches the arterial wall previously destructured by the decidual natural killer cells [44], and replaces the elastic lamina and the muscular wall with an amorphous fibrinoid material that escapes vasomotor control [45]. Additionally, it penetrates the lumen of these vessels, giving rise to the endovascular trophoblast that extends proximally [36].

In the early stages of placental development, the endovascular trophoblast completely occludes the lumen of the terminal region of the spiral arteries, preventing perfusion of the villous space. When this occlusion disappears around the tenth week of pregnancy, maternal blood and chorionic villi enter direct contact and give rise to the hemochorial placenta [45].

The initial development of the placenta and embryo – before 10 to 12 weeks of pregnancy, and therefore, of the perfusion of the chorionic villi – occurs in a low partial pressure of oxygen environment due to growth factors and proteins produced by the endometrial glands [46]. In pathological situations in which there is an increase in the partial pressure of oxygen at this stage (by early or uncoordinated perfusion of this villous space), increases of oxidative stress at the placenta and embryo causes villous atrophy and miscarriage [47].

Both endovascular and interstitial migration of the extravillous trophoblast is necessary and interdependent [48]. In normal pregnancy, migration extends to the inner third of the myometrium [36]. The modification of the artery segment immediately proximal to the endomyometrial junction is significant, to the point that this area of the myometrium is considered a specialized tissue. Outside of pregnancy, vasoconstriction of the spiral arteries at this level limits the amount of blood lost during menstruation. However, modification of this segment during pregnancy ensures adequate perfusion of the villous space. When trophoblastic invasion does not reach the endomyometrial junction, it is insufficient or superficial; it increases the risk of miscarriage, pre-eclampsia, and intrauterine growth restriction [49].

From a clinical perspective, altered remodeling of the spiral arteries of the uterus is assessed indirectly by uterine artery Doppler velocimetry. The persistence of a protodiastolic notch and a pulsatility index above the 95th percentile from the 11th to 14th week are risk markers of pre-eclampsia [50].

Abnormal perfusion of the intervillous space occurs due to this defective remodeling, resulting in villous damage and placental dysfunction. The dysfunctional and hypoxic placenta releases soluble tyrosine kinase-1 (sFlt-1) and endoglobin molecules into the maternal circulation, which are associated with the clinical manifestations of pre-eclampsia (hypertension, proteinuria, coagulation disorders, liver dysfunction, systemic inflammatory response, and marked endothelial dysfunction) by decreasing the bioavailability of proangiogenic factors such as PlGF [51]. An increased sFlt-1/placental growth factor ratio is directly associated with the development of pre-eclampsia. This ratio helps identify patients at very high risk that require imminent termination of pregnancy because of an adverse maternal and neonatal prognosis [52-54].

Human leukocyte antigen expressed on the trophoblast modulate uterine natural killer cell activity

Selective expression of HLA molecules on the trophoblast surface is essential for pregnancy development [55]. Under normal conditions, the syncytiotrophoblast lacks class I and class II HLA molecules [56-58]. In contrast, the extravillous trophoblast expresses only class I molecules (HLA-C, HLA-E, HLA-F, and HLA-G) [59,60].

The absence of class I and class II molecules (HLA-DP, HLA-DQ, and HLA-DR) on syncytiotrophoblast and class II molecules on the cytotrophoblast prevent the T-cell-mediated alloimmune response against HLA of paternal origin [61].

The trophoblast's expression of class II molecules is considered abnormal and associated with various obstetric pathologies. For example, it has been observed that syncytiotrophoblast and cytotrophoblast from pregnancies terminated in unexplained abortion between 12- and 24-weeks express HLA-DR molecules, which are not present in placentas from healthy controls (pregnancies terminated by voluntary termination) [62]. In the syncytiotrophoblast of placentas from term pregnancies, the expression of HLA-DR, DP, and DQ molecules is confined to areas of villitis, much more frequent in women with a history of secondary repeat abortions [63]. Additionally, HLA-DR expression has been demonstrated in the syncytiotrophoblast of women with pre-eclampsia [61] and within the endovascular cytotrophoblast of women with pre-eclampsia, which is associated with impaired spiral artery remodeling [64].

In contrast, the expression of class I molecules on the trophoblast is considered essential for gestation. Genetically, these can be oligomorphic (HLA-E and HLA-G) or polymorphic (HLA-C), and their expression is essential for the extravillous trophoblast. The binding of these molecules to specific receptors on uterine natural killer cells generates inhibitory or activating signals that control cytotoxicity and promote the secretion of cytokines necessary for placentation during the first half of pregnancy [65].

HLA-E expressed by the extravillous trophoblast is recognized by NKG2A/NKG2C receptors of uterine natural killer cells [66], and the inhibitory signal resulting from this interaction predominates over most activating signals [67]. Since the trophoblast does not express HLA-E after the seventh week of pregnancy, the function of this molecule appears to be restricted to implantation and early embryo development [60]. Embryonic HLA-G, in turn, can interact with at least two types of receptors on uterine natural killer cells: LILRB1 [68] and KIR2DL4 [69,70]. By binding to these receptors, HLA-G stimulates the secretion of cytokines such as interleukin-6, interleukin-8, and tumor necrosis factor [70] that promotes immune tolerance at the maternal-fetal interface [71,72]. The extravillous trophoblast is one of the few tissues that express HLA-G under physiological conditions [73]. Likewise, its interaction with KIR2DL4 (present in almost all individuals) is considered essential for the reproductive process. However, in 2004 Gómez-Lozano et al. reported a multiparous woman with no history of obstetric complications and absence of 2DL4. Like in other areas of the immune system, this case suggests redundant mechanisms in decidual natural killer cells that can compensate for alterations or occasional receptor losses [74].

Various KIRs on uterine natural killer cells recognize the HLA present on the extravillous trophoblast at the maternal-fetal interface [66]. These are the only polymorphic class I molecules, and different allotypes have been associated with diverse reproductive outcomes depending on the KIR repertoire presented by uterine natural killer cells [75]. KIRs are capable of recognizing all HLA-C and a small variety of HLA-A (A3/11) and HLA-B (Bw4) allotypes [76]. Because the extravillous trophoblast does not express HLA-A or HLA-B, as mentioned above, this interaction will not be the subject of this review.

The binding between HLA-C with the KIR receptor is determined by the dimorphism of an amino acid at position 80 of the α-1 domain of the HLA-C molecule. Molecules containing asparagine at position 80 form the C1 epitope, and those containing lysine corresponds to the C2 epitope [77,78]. Based on this, more than 1000 described HLA-C alleles could be classified into two groups: C1 and C2, respectively.

Although this interaction is not specific, KIR 2DL2 and 2DL3 recognize with higher affinity C1 epitopes while KIR 2DL1, 2DS1, and 2DS4 recognize with higher affinity the C2 epitope. The binding of C1 alleles and KIR 2DL2 and 2DL3 produces a weak inhibitory signal in decidual natural killer cells, whereas binding of C2 to KIR 2DL1 results in a strong inhibitory signal [55]. In contrast, C2 binding to KIR 2DS1 [37], 2DS4 [79], and possibly some alleles of 2DS5 present in the centromeric region of African, not of European origin [80], activate decidual natural killer cells.

Inhibition of uterine natural killer cells resulting from the interaction between HLA-C2 and KIR2DL1 decreases degranulation and the release of cytokines (interleukin-8, vascular endothelial growth factor, PGF and CXCL10) that influence extravillous trophoblast development [25]. In contrast, KIR2DS1- and KIR2DS4-mediated activation induces increased degranulation of uterine natural killer cells [81] and increased secretion of GM-CSF and tumor necrosis factor-α [79,82]. Although KIR 2DL1 and 2DS1 recognize the same ligand (HLA-C2), the affinity of the former is 3.5-fold higher [83]. A similar phenomenon occurs with KIR 2DL2, 2DL3 and 2DS2, and HLA-C1. Although these receptors recognize the same ligand, the binding affinity of 2DL2 is higher than that of 2DL3. In contrast, the binding of HLA-C1 to 2DS2 in many studies has been even lower or could not be demonstrated [83]. This phenomenon implies that in decidual natural killer cells expressing both receptors, the inhibitory effect predominates over the activating one.

Finally, regardless of the epitope presented to natural killer cells, HLA-C molecules are expressed differently on the cell surface. Their

expression is modulated by structural differences that condition everything from their transcription to their ability to bind intracellular peptides [84].

The magnitude of the expression of HLA molecules on the cell surface modifies the intensity of the immune response [84]. Their expression has been related to the evolution of HIV infection and the risk of progression to AIDS [85], the risk of developing Crohn's disease [86], and the prognosis of hematopoietic progenitor transplantation. In the latter, the best tolerated human HLA mismatches corresponded to the least expressed alleles on the cell surface, including those that may be more immunogenic by the degree of mismatch with the recipient's human HLA [87]. According to studies performed by Apps and collaborators [85], the most expressed alleles were C*14, C*01, and C*12 among the C1 group, and C*18, C*06, and C*15 among the C2 group; while the least expressed were C*16, C*08, C*03, C*07 among C1 group and C*04, C*02, C*05 and C*17 among C2 group.

HLA-C molecules' expression has been similar in the different ethnic groups studied [85].

KIR receptors: structure, genetic and functional organization

KIRs are membrane proteins expressed on specific subpopulations of natural killer cells located in the endometrium/decidua, peripheral blood, and some T lymphocytes [88].

The KIR loci comprise a set of 14 genes and two pseudogenes, located in a continuous sequence of approximately 150 kilobases, within the leukocyte receptor complex on chromosome 19 (19q13.4) [88]. Among these, eight inhibitory KIRs (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2 and 3DL3), six activating KIRs (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1) and two pseudogenes (2DP1 and 3DP1) are distinguished.

Each receptor consists of two or three extracellular immunoglobulin-like domains (KIR2D and KIR3D, respectively) coupled to a transmembrane domain and a cytoplasmic region (cytoplasmic stalk), which can be either short (S) or long (L). In general, long cytoplasmic stalks are associated with tyrosine-based inhibitory immunoreceptors and short stalks with activating intracellular signaling molecules of the DAP12 or ITAM type [89]. The exception to this rule is KIR2DL4, that unlike other KIRs expressed on the cell surface, predominantly localizes in endosomes and interacts with HLA-G via trogocytosis [72]. Moreover, although it has a long cytoplasmic stalk, it can transmit activating signals – and far from activating the cytotoxicity of uterine natural killer cells – stimulating the secretion of cytokines that favor placental development [90].

In turn, each KIR is encoded by an individual gene composed of eight or nine exons corresponding to each of the receptor domains. In 3D KIRs (e.g., 3DL1 and 3DL2), exons one and two encode the signal peptide; exons three, four, and five the extracellular immunoglobulin-like domains (D0, D1, and D2); exons six and seven the extracellular and transmembrane portions of the stalk, respectively; and exons eight and nine the intracytoplasmic portion [89]. A similar pattern occurs in 2D KIRs; however, two groups are distinguished by their KIRs. Group one KIRs (KIR2DL1, 2DL2, 2DL3, 2DS1, 2DS2, 2DS3, 2DS4, and 2DS5) lack the D0 domain and have a D1 type immunoglobulin-like domain at the distal (N-terminal) end. The latter is encoded by exon 4 of the corresponding KIR gene. In contrast, group 2 (KIR2DL4 and 2DL5) lacks the D1 domain, and the distal domain corresponds to a D0 domain encoded by exon 3 of the gene [88].

Genetic variations at different levels can modify KIR proteins characteristics, including their expression on the cell surface, their affinity for HLA class I molecules, and intracellular signaling mechanisms.

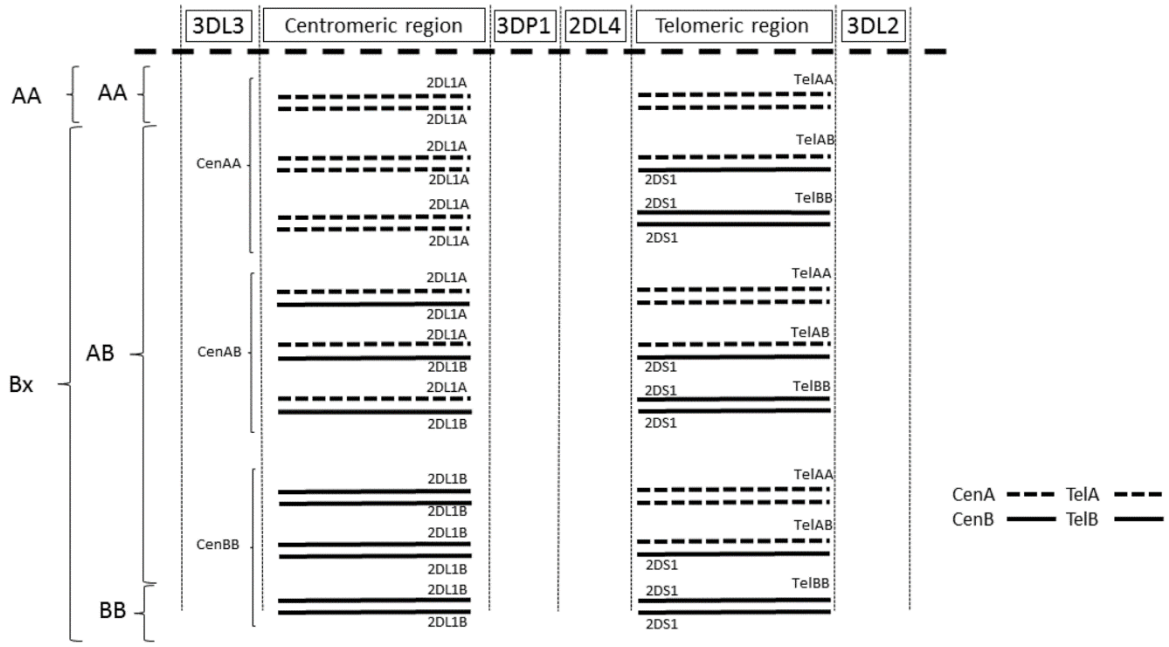
KIR genes are arranged in haplotypes with variable gene content, delimited by genes conserved in most individuals and considered framework genes. These genes are located at the centromeric and telomeric ends of each haplotype and in the central region. KIR haplotypes' centromeric end is defined by 3DL3, the telomeric end by 3DL2, and the central region by 3DP1 and 2DL4 [91]. The distance separating 3DP1 and 2DL4 (5 to 14 kilobases) is more extensive than that the distance with the rest of the KIR genes (3 kilobases) [92]. The latter is the most frequent point of genetic recombination. It is used to define two zones within the haplotype, also called motifs: a centromeric motif between 3DL3 and 3DP1 and a telomeric motif between 2DL4 and 3DL2. Most haplotypes in the population can be explained by recombining these centromeric and telomeric motifs [91].

The Human Genome Nomenclature Committee of the World Health Organization distinguishes two haplotypes: the A and the B. Haplotype B is characterized by KIR2DL5, 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1; whereas haplotype A by the absence of these genes [93]. Based on their gene content, haplotype A would consist of a defined gene content (KIR3DL3-2DL3-2DP1-2DL1-2DL1-3DP1-2DL4-3DL1-2DS4-3DL2) [94]; and haplotype B, of variable gene content, characterized by the absence of KIR2DL3, 3DL1, and 2DS4 [95].

Excluding the KIR *framework*, haplotype A results from a centromeric motif A (cen-A or cA), consisting of KIR2DL3-2DL1-2DP1, and a telomeric motif A (tel-A or tA), consisting of KIR3DL1-2DS4. Similarly, a centromeric B motif (cen-B or cB) and a telomeric B motif (tel-B or tB) are identified in the B haplotype. The B motifs (unlike the A motifs) have variable gene content. In cen-B motifs, KIR2DS2 and 2DL2 are present, accompanied or not by KIR2DL1 and 2DP1, and in tel-B motifs, by KIR3DS1 and 2DS1. Although the KIR 2DL5, 2DS3, and 2DS5 genes are unique to haplotype B, both cen-B and tel-B motifs may be present [93]. Because an individual inherits one haplotype from each parent, individuals with genotype A (AA) carry two complete A haplotypes (two cen-A motifs, and two tel-A motifs) and individuals with genotype B (BB) carry two complete B haplotypes (two cen-B and two tel-B motifs). However, in-

dividuals with genotype AB can carry any combination of centromeric and telomeric A and B motifs (Figure 1), such that two individuals with genotype AB can have very different gene content.

Figure 1. Representation of the different combinations of KIR genes.



KIR: Killer inmunoglobin-like receptors
Source: Prepared by the authors of this study.

The KIR region is one of the most polymorphic regions of the leukocyte receptor complex. In addition to the variability generated by the different combinations between centromeric and telomeric motifs, there is also an allelic polymorphism. The highest allelic diversity has been recorded in East Africa and appears to be decreasing as populations move away from this geographic region. The highest diversity region corresponds to the Ga-Adangbe population (Ghana, East Africa), while the lowest diversity has been recorded in populations native to the Americas, such as the Yucpa (Venezuela) [77]. Allelic polymorphism is especially important in haplotype A genes. However, cen-B or tel-B motifs with the same gene content will likely have the same allelic content [91].

In the European Bioinformatics Institute database, 977 alleles have been recorded so far for the 16 KIR genes. The most polymorphic gene is 3DL3 (164 alleles described), and the least polymorphic are KIR 2DS3 and 2DS1 (16 alleles recorded for each). For KIR 2DL1, 64 alleles have been described, while for KIR2DL2, KIR 2DL3, and KIR 2DS4 33, 59, 37 alleles have been described, respectively (www.ebi.ac.uk/ipd/kir/). The polymorphism of 2DL1 and 2DL2/3 (both inhibitory receptors for HLA-C2 and HLA-C1) contrasts with how conserved KIR2DS1 (activating receptor for HLA-C2) remains [96].

Regarding human reproduction, the study of the different combinations between HLA and KIR is performed by excluding framework genes and taking into account only those KIRs that interact with HLA-C – which is the only polymorphic HLA molecule expressed by the extravillous trophoblast.

Following this premise, in haplotype A, two centromeric inhibitory receptors (KIR2DL3 and 2DL1) and one telomeric activating receptor (KIR2DS4) are present. And in haplotype B, two centromeric inhibitory receptors (KIR2DL2 and 2DL1) and one telomeric activating receptor (KIR 2DS1) are identified.

Although the configuration of both haplotypes is similar from a theoretical point of view, functionally, they behave differently. The main variables that explain this difference are the expression of KIR receptors on the surface of decidual natural killer cells, the affinity for their ligands, and their intracellular signaling mechanisms. These,

in turn, are determined by the genetic content and conditioned by allelic polymorphism [97]. In all, haplotype A is characterized by a predominance of inhibitory KIRs and haplotype B by activating KIRs.

In decidual natural killer cells from individuals with haplotype A, the binding of HLA-C2 to KIR2DL1 produces a strong inhibitory signal, which cannot be counteracted by stimulation of 2DS4 (the only activating receptor present in this haplotype). Stimulation of KIR2DS4 favors trogocytosis, which is the primary mechanism of HLA-G acquisition in natural killer cells [98]. However, in the European population, the KIR2DS4 alleles most frequently identified in the population (KIR2DS4*003/004/006) are characterized by a 22 base pair deletion that introduces a mutation in its reading frame and synthesizes a soluble protein with a single intact immunoglobulin-like domain that does not bind HLA-C (KIR2DS4del) [79]. The binding of HLA-C1 antigen to KIR2DL3 also produces an inhibitory signal on the decidual natural killer cell. However, this is much weaker and has so far not been considered relevant.

In contrast, in decidual natural killer cells from haplotype B individuals, the binding of HLA-C2 to KIR2DS1 produces a strong activating signal that counteracts the effect of KIR2DL1-mediated inhibition. Similar to haplotype A with KIR2DL3, the binding of HLA-C1 with KIR2DL2 produces a weak inhibitory signal on decidual natural killer cells [55].

Another important aspect contributing to the functional difference between these two haplotypes is allelic polymorphism. Although not all KIR alleles described have been completely sequenced, KIR2DL1 alleles at Cen A motifs generally produce a higher inhibitory response. They have higher avidity for HLA-C2 than those secreted at Cen B motifs. In contrast, KIR2DL2/3 alleles secreted at Cen A motifs produce a lower inhibitory response and have lower avidity for HLA-C1 than those secreted at Cen B motifs [96].

In the European population, the KIR2DL1 alleles most frequently associated with haplotype A are KIR2DL1*003, *002, and *001 and can be grouped as KIR2DL1A. On the other hand, KIR2DL1*004 is much more frequent in haplotype B and can be designated 2DL1B [97]. The latter has lower avidity for HLA-C2 and produces an inhibitory signal of lower intensity than KIR2DL1*003 [99].

In individuals carrying both alleles (KIR2DL1A/B), it has been shown that the population of natural killer cells (peripheral blood and decidual) expressing KIR2DL1A is significantly larger than that expressing KIR2DL1B and that only a small population co-expresses both alleles. In natural killer cells simultaneously expressing KIR2DL1A and KIR2DL1B, the inhibitory response is greater than those expressing only KIR2DL1B but less than those expressing only KIR2DL1A. The latter finding suggests that the magnitude of the inhibitory effect is related to the number of copies of KIR2DL1A present in the individual's genotype [55]. Thus, carriers of two CenA motifs would have two copies of 2DL1A (the maximum gene dose).

The effector capacity of the natural killer cell depends on the repertoire of receptors it expresses on its surface. Unlike other immune system cells – such as T and B lymphocytes that can generate clonotypic receptors by genetic rearrangements and specifically recognize a wide variety of antigens – natural killer cells apply their cytotoxic effect directly or through an antibody-mediated response to a wide variety of stimuli. Their rapid and relatively nonspecific response is essential for an early antiviral and antitumor response. This response depends on tightly regulated adaptation mechanisms and prevents self-destructive effector response [100]. This control is achieved through the expression of a series of inhibitory receptors (such as KIRs) capable of recognizing class I HLA molecules expressed in all cells (except red blood cells) and allows the natural killer cell to recognize "*self*" from "*non-self*".

Natural killer cells acquire receptor repertoire stochastically, depending on the genetic content. This mechanism generates multiple natural killer cell subpopulations with different receptor combinations (phenotype) within the same individual [101]. However, the pool of receptors is modeled as they interact with their specific ligands (HLA molecules) in the rest of the organism. The process by which the natural killer cell models its definitive repertoire of receptors, thereby developing its functional competence, is known as education. This process allows the natural killer cell to acquire its "license to kill" [102].

In general, natural killer cells with higher cytotoxic capacity are characterized by potent inhibitory receptors that limit self-destruction. In contrast, natural killer cells expressing weak inhibitors or with low affinity for their ligand have a lower cytotoxic capacity. Consequently, potent inhibitory receptors are determinants for the education of natural killer cells [101].

During differentiation, peripheral blood natural killer cells express first the NKG2A receptor (which, as previously mentioned, exerts a potent inhibitory effect on the natural killer cell) and subsequently KIRs [101]. Suppose the KIR receptors expressed by the cell encounter their specific ligand (constitutive HLA-C molecules) and have an intracellular signal strong enough to stop the cytotoxic response. In that case, this receptor is conserved, and the cell ceases to express NKG2A. Additionally, it has been observed that natural killer cells express KIR2DL2, 2DL3, and 2DS2 earlier than KIR2DL1 and 2DS1. Therefore, the latter's expression in the absence of NKG2A has been considered a sign of differentiation [103,104].

In peripheral blood natural killer cells, the HLA-C conditions the KIR repertoire. In contrast, endometrial natural killer cells exhibit a unique repertoire of receptors independent of HLA. Additionally, while a similar percentage of endometrial natural killer cells and peripheral blood natural killer cells express KIR2L1/S1, the percentage of endometrial natural killer cells expressing NKG2A, LILRB1, KIR2DL2/L3/S2, KIR2DL3 is significantly higher. The expression of NKG2A on these cells could favor the immunomodulatory effect of HLA-E and seems to play a vital role in early pregnancy [66]. The repertoire of receptors present on endometrial natural killer cells suggests that these are immature cells that reach their final differentiation once pregnancy occurs in the presence of embryonic HLA-C.

In the study of decidua between 8 and 12 weeks, obtained from women undergoing voluntary termination, it has been observed that more than 90% of decidual natural killer cells express NKG2A. However, KIR expression in decidual natural killer cells is conditioned by maternal but not fetal HLA-C. The percentage of decidual KIR2DL1+ natural killer cells in women with at least one C2 epitope is significantly lower than in C1/C1 women. In contrast, the percentage of decidual KIR2DL3+ natural killer cells is higher in C1/X women than in C2/C2 women [105].

Knowledge of the decidual natural killer cell education process is essential to explain how different combinations of fetal HLA-C and maternal KIR genotype may contribute to the development of pregnancy.

Concerning evidence obtained so far, KIR AA women are at increased risk of developing pre-eclampsia, intrauterine growth retardation, and miscarriage when pregnant with fetuses expressing the C2 epitope. This effect appears to be especially important when the fetal C2 epitope is of paternal origin (when the mother is C1C1 or when the embryo is C2C2). Certain combinations between KIRs and HLA-C inhibiting decidual natural killer cells may prevent adequate trophoblast invasion and contribute to the subsequent development of pre-eclampsia, intrauterine growth retardation, or gestational loss [17,37]. In cases of women with KIR AA undergoing assisted reproduction with sperm or oocyte donors, authors suggest selecting HLA-C1/C1 donors to avoid pre-eclampsia as much as possible [55].

Adverse outcomes in this situation are attributed to the interaction between the embryonic HLA-C (expressed on the extravillous trophoblast) and KIR2DL1. This binding inhibits decidual natural killer cells and decreases the secretion of proangiogenic cytokines within the placental bed. The deficient decidual natural killer cell activity results in a superficial trophoblastic invasion, impaired spiral arteries remodeling, and inappropriate blood delivery to the intervillous space, which eventually disrupts villous development. Consequently, the placenta releases chemical mediators responsible for the systemic alterations, characteristic of pre-eclampsia. And finally, massive involvement of the chorionic villi causes placental insufficiency and compromises fetal development.

In women with AB and BB genotypes, the presence of KIR2DS1 decreases the risk of complications. In this situation, the binding of fetal HLA-C2 and KIR2DS1 activates decidual natural killer cells counteracting the inhibitory effect of KIR2DL1 [106]. In the European population, the KIR2DL1 allele most frequently associated with CenB is KIR2DL1*004. As previously described, this allele encodes a less receptor expression, a lower affinity for the HLA-C2, and exerts a weaker inhibitory effect than the alleles associated with the CenA motifs (collectively called KIR2DL1A) [97].

In the light of new high-resolution phenotypic and genetic evidence, it has been shown that the KIR2DL1A allele is specifically associated with an increased risk of pre-eclampsia. Furthermore, the risk of pre-eclampsia was directly related to the KIR2DL1A gene dosage, being higher in women with two KIR2DL1A alleles (CenAA) [97].

In women with BB genotype, characterized by the absence of KIR2DL1A and the presence of two KIR2DS1 alleles, the HLA-C2 activates the decidual natural killer cell favoring deep implantation and placental development. It has been observed that in women with the KIR BB genotype, heavier fetuses are more frequent, leading to increased obstetric complications during labor [106].

Without timely medical intervention, both superficial invasion (which may be associated with miscarriage, pre-eclampsia, and intrauterine growth restriction) and deep invasion of the extravillous trophoblast (which may be associated with increased fetal weight and possibly placental accreta) have been associated with increased maternal and perinatal morbidity and mortality. These mechanisms and their outcomes could have modulated the population distribution of HLA-C and KIR throughout evolution [107].

Conclusions

Implantation and placental development depend on the communication between the embryo and the maternal immune system at the maternal-fetal interface. The selective expression of HLA molecules allows extravillous trophoblast to interact with receptors that modulate decidual natural killer cells. This interaction reduces natural killer cells' cytotoxicity and favors proangiogenic cytokine secretion that promotes placental development. Ultimately, adequate remodeling of spiral arteries and good irrigation of the villous space depends on this interaction.

HLA-C molecules and KIR genes are highly polymorphic. However, they have retained the ability to recognize these molecules throughout evolution. The interaction between embryonic HLA-C and KIR is considered critical for reproduction. The fact that KIR2DS1 is one of the most evolutionarily conserved alleles reinforces this idea. Binding to embryonic HLA-C2 activates the decidual natural killer cell, promoting placental development and decreasing the risk of obstetric complications associated with KIR2DL1A (the main inhibitory receptor).

Studying the HLA-C/KIR interaction through molecular biology techniques and its incorporation into diagnostic and therapeutic algorithms could allow fundamental advances in preventing pregnancy complications. Likewise, it could improve the outcome of assisted reproduction techniques. In all, these are the main objectives of immunoperinatology and reproductive immunology.

Notes

Contributor roles

ERF: original draft preparation, review and editing, visualization. MFA: conceptualization, review and editing, and visualization. NSIM: original draft preparation, review and editing, visualization, and project management. AO: methodology, preparation of the original draft, review, and editing, and visualization. IC: review and editing, and visualization. MAH: con-

ceptualization, methodology, validation, review and editing, and visualization. SSR: conceptualization, methodology, validation, preparation of the original draft, review, and editing, visualization, supervision, and project management.

Competing interests

The authors declare that they have no competing interests and have completed the IMCJE form. In addition, they declare that they have no financial relationships with organizations that could have an interest in the published article and have no other relationships or activities that could influence the article.

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Ethics

Due to the nature of the study, it did not require evaluation by an ethics committee.

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