Maternal-fetal HLA compatibility and trophoblast-immune interactions in healthy and preeclamptic pregnancy: elegance in complexity

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Introduction

The maternal-fetal interface: a unique immunological challenge

Pregnancy represents a striking immunological paradox. Despite the semi-allogeneic nature of the fetus and placenta, carrying both maternal and paternal antigens, they are able to grow without complications. In contrast, exposure to non-self-antigens in other situations, such as infections, organ transplants, or cancer, typically provokes an immune response aimed at eliminating the foreign entities. Interestingly, the foreign paternally inherited antigens do not elicit such an immune reaction during pregnancy. This phenomenon, first articulated by Medawar in 1953, challenged classical immunological principles and spurred decades of investigation (1). The hypotheses that the fetus is antigenically immature, the uterus is immune privileged, or maternal immunity is generally suppressed, have since been replaced by a more nuanced understanding: pregnancy involves a highly regulated state of immune adaptation and modulation (2).

At the core of this adaptation is the maternal-fetal interface, where fetal trophoblast cells encounter maternal immune cells. Rather than passive tolerance, a complex interplay unfolds involving local immune cell recruitment, specialized phenotypes, and active immunomodulation. Immune activation remains possible, especially to defend against pathogens, yet immune tolerance is promoted to prevent immune-mediated damage to the fetus. Importantly, the recognition of fetal antigens is actually essential for proper placentation and maintenance of pregnancy (3). Aberrant immune responses, disrupting the delicate balance between activation and regulation, are associated with severe pregnancy complications, like preeclampsia (4).

This dissertation explores two crucial immunological elements that are essential for a successful pregnancy. Both play a key role in the pathophysiology of preeclampsia and may hold predictive value for it: (1) maternal-fetal HLA compatibility, and (2) the generation of a tolerogenic immune environment by fetal trophoblasts.

Placental development and trophoblast differentiation

In preparation for pregnancy, the human endometrium undergoes marked structural and immunological changes that support implantation and early placentation. Following ovulation, rising progesterone levels drive the transition of the endometrium into a receptive, secretory state and initiate differentiation into the decidua even before embryo implantation (5). This process is paralleled by dynamic shifts in the composition and function of immune cells within the uterine mucosa. In particular, decidual natural killer cells (dNK, also referred to as uterine NK), which represent the dominant lymphocyte population in the late secretory phase, expand in number and have a specialized $CD56^{high}$ phenotype adapted to the decidual environment (6). Dendritic cells and macrophages localize around the implantation site and contribute to implantation success (5). These changes occur in concert with the

recruitment and activation of regulatory T cells (Tregs), which are essential for establishing tolerance to the semi-allogeneic fetus (7). While hormonal changes related to the menstrual cycle contribute to immune responses at the implantation site, pre-conceptional exposure to seminal fluid may also promote beneficial responses that facilitate implantation. The initial increase in Tregs may be triggered by antigen presenting cells (APCs) in the vaginal or endometrial lumen that encounter paternal antigens in seminal fluid (8, 9).

Immune interactions between the mother and the fetus mainly occur at the maternal-fetal interface, specifically the placenta and decidua. The placenta is the key organ supporting fetal growth and maternal-fetal exchange. It derives at the site of the implantation of the fetal blastocyst into the maternal decidua (Figure 1)(10). The blastocyst comprises two distinct cell populations; the trophectoderm, which encapsulates the blastocyst and gives rise to the fetal cells of the placenta (trophoblasts), and the inner cells mass, which gives rise to the embryo and the visceral endoderm. The stem cells of the trophectoderm give rise to the villous trophoblasts, also called cytotrophoblasts (CTB). CTBs form villi within lacunae through which the maternal blood will flow. The outer layer differentiates into the multi-nucleated fused syncytiotrophoblast (STB). Therefore, the STB is in direct contact with the maternal blood. The STB performs various biological functions, including facilitating gas and nutrient exchange between the mother and fetus, secreting various hormones to maintain pregnancy, and acting as the primary maternal-fetal barrier against pathogens. The STB also plays a key role in regulating maternal-fetal immune tolerance through contact with the maternal peripheral blood that passes by, which consists of leukocyte populations other than the specialized decidual maternal immune cells. It does so by secreting immunomodulatory molecules, releasing extracellular vesicles, and controlling complement activation (11).

Extravillous trophoblasts (EVT) arise from CTB and invade the maternal decidua interstitially and endovascularly (12). Endovascular EVT displace endothelial cells and degrade the muscular wall of spiral arteries, preventing vasoconstriction and promoting stable perfusion. Remodeled spiral arteries by EVT create low-resistance, high-capacitance (expandable, high-volume) blood flow in the placenta (13). This transformation includes a complex cascade of processes that includes local maternal immune activation, particularly by specialized dNK. Disruption of spiral artery remodeling is a hallmark of preeclampsia (14, 15).

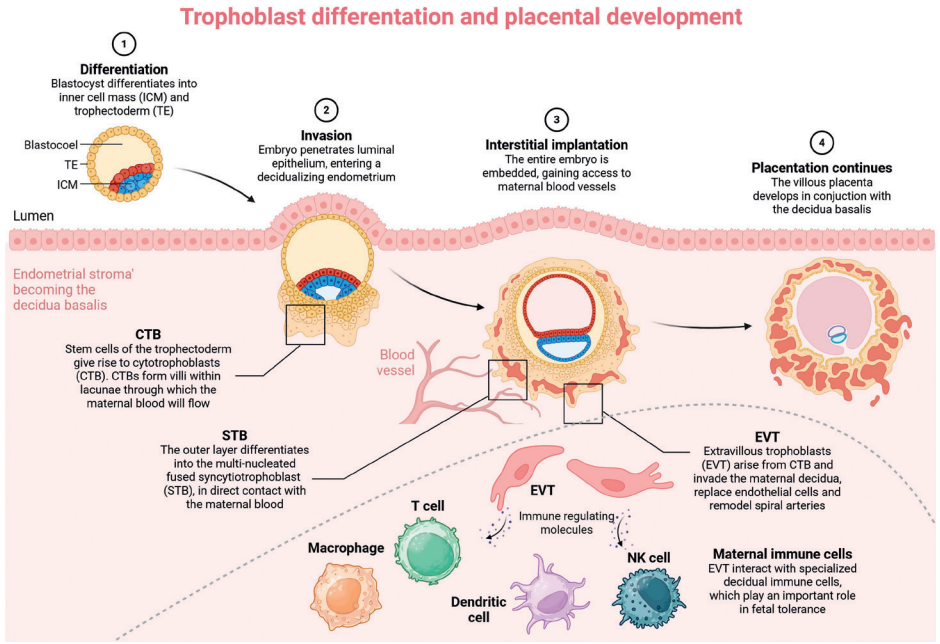

Figure 1. Trophoblast differentiation and placental development. The blastocyst differentiates into the inner cell mass and the trophectoderm (1), before it invades the decidua basalis (2) where implantation (3) and subsequent placentation (4) occurs. The trophectoderm-derived trophoblast lineage differentiates into distinct subtypes with specialized roles in placental development and immune regulation. Cytotrophoblasts (CTB) form the proliferative villous core and give rise to both the multinucleated syncytiotrophoblast (STB) and extravillous trophoblasts (EVT). The STB forms the outermost villous layer in direct contact with maternal blood, where it facilitates nutrient and gas exchange, hormone secretion, and immunomodulation. EVTs invade the maternal decidua through infiltration of the decidual stroma and remodeling spiral arteries by displacing endothelial and smooth muscle cells. This vascular transformation ensures low-resistance, high-capacitance maternal blood flow into the placenta. Maternal immune cells interact with invading EVTs and contribute to regulating trophoblast invasion and vascular remodeling. Together, these trophoblast subtypes orchestrate placental development while shaping maternal–fetal immune tolerance (2, 10, 16).

The fetus itself is never in direct contact with maternal tissues. Instead, the interactions between fetal and maternal cells occur at defined sites collectively referred to as the maternal-fetal interface. There are three primary locations of contact. First, the decidua basalis represents the maternal side of the placenta where the invading EVT interact with specialized maternal immune cells. Second, the decidua parietalis is where the maternal lining of the uterus comes into contact with the fetal membranes, particularly the outer chorion containing non-invading trophoblasts. Third, the STB forms the outer layer of the chorionic villi and directly contacts the maternal blood during utero-placental circulation. Additionally, fetal cells and cell-derived particles that enter the maternal bloodstream may represent a fourth, systemic point of contact between maternal and fetal compartments. Contact sites in the decidua basalis and the intervillous space together constitute the

functional placenta. Each of these interfaces has a distinct cellular composition and immunological profile, which change dynamically throughout gestation.

Maternal immune cell populations at the maternal-fetal interface

The decidua is populated by a dynamic population of specialized local immune cells that differ in quantity and function throughout pregnancy (2, 16-19). dNK cells and myeloid cells are abundant during the first trimester, while T cell numbers relatively are low. Throughout gestation, the dNK cell compartment decreases, the T cell compartment increases and the myeloid compartment remains stable. These cells adapt to the local environment and interact with fetal trophoblasts in highly regulated ways to support pregnancy.

Decidual NK cells

Decidual NK cells are central to pregnancy by regulating placentation, maintaining immune tolerance, and therefore supporting fetal development. dNK cells represent up to 70% of leukocytes in the first trimester decidua and exhibit phenotypes distinct from peripheral NK cells (pNKs) (20). With their $CD56^{bright}CD16^{-}$ phenotype as a key feature, dNK are only weakly cytotoxic and do not normally kill trophoblast cells (21). The majority expresses unique receptors like Killer-cell Immunoglobulin-like Receptors (KIRs), LILRB1 and CD94/NKG2A (6). Most dNK cells secrete a tailored array of cytokines and growth factors, such as Interferon-gamma (IFN-γ), Vascular endothelial growth factor (VEGF), and Granulocyte-macrophage colony-stimulating factor (GM-CSF), which promote trophoblast invasion, spiral artery remodeling, and tissue support crucial for placental development (6). dNK cells are less cytotoxic than pNKs and instead contribute to immune tolerance at the maternal-fetal interface by dampening local inflammation and interacting with other immune cells, including regulatory T cells, to prevent anti-fetal immune responses (6, 20, 22).

The education and function of dNK cells are critically dependent on the inhibitory receptor CD94/NKG2A, which engages non-classical human leukocyte antigen (HLA)-E on trophoblasts to “educate” dNK cells for optimal cytokine secretion, maternal vascular adaptation, and fetal growth (22). Disruption of this pathway in both mice and humans leads to impaired dNK function and poor pregnancy outcomes, such as fetal growth restriction and preeclampsia (22).

The KIR expression of dNK is specifically adapted for recognizing fetal trophoblast HLA-C, enhancing their ability to mediate appropriate immune responses that support vascular remodeling (23). Interestingly, the KIR and HLA-C expression during pregnancy is location- and gestation-dependent, with the highest per-cell intensity and frequency of HLA-C-specific KIR in the first trimester decidua (24). Maternal KIRs are highly polymorphic and segregate into two major haplotypes, designated A and B (25). Haplotype A encodes

primarily inhibitory receptors, whereas haplotype B contains additional activating receptors. A pregnant individual may therefore carry a KIR AA genotype (lacking activating KIRs) or an AB/BB genotype (containing one to ten activating KIRs). Activation through these KIRs is required for optimal dNK cell function, including adequate trophoblast invasion and spiral artery remodeling. The ligands for KIRs are HLA-C molecules expressed by EVT, making the maternal KIR–fetal HLA-C interaction a central determinant of placental development. Fetal HLA-C molecules are divided into two groups based on a dimorphism at position 80 of the α1 domain (26). Group 1 HLA-C (C1) allotypes bind inhibitory KIR2DL2/3, whereas group 2 HLA-C (C2) allotypes bind both inhibitory KIR2DL1 and activating KIR2DS1. HLA-C1 allotypes bind inhibitory KIR2DL2/3, while HLA-C2 allotypes bind both inhibitory KIR2DL1 and activating KIR2DS1. EVT express both paternally and maternally inherited HLA-C surface proteins. The combination of maternal KIR and fetal HLA-C genotypes influences dNK activation, which in turn affects placental development that is reflected in pregnancy outcome (3, 27-31). Increased dNK activation leads to increased production of GM-CSF and increased migration of a trophoblast cell line in vivo, suggesting that this interaction may lead to enhanced placentation (32). Conversely, pregnancies in mothers with a homozygous KIR haplotype A (KIR AA) are at an increased risk of preeclampsia, particularly when the fetus inherits more HLA-C group 2 (C2) alleles than the mother (29). This unfavorable combination of maternal KIR AA and a paternal HLA-C allele bearing a C2 epitope is also associated with fetal growth restriction (FGR) and recurrent pregnancy loss (RPL) (28). Meanwhile, the activating KIR2DS1 receptor in combination with HLA-C2 is associated with higher birthweight (27). Therefore, NK cell allorecognition appears to balance fetal growth needs with maternal health and reproductive fitness.

T cells

T cells are a central component of the adaptive immune system at the maternal-fetal interface. They comprise both conventional T cells (Tconvs) and Tregs, with $CD4^+$ and $CD8^+$ subsets present in decidua, but playing distinct roles. Tconvs retain the potential to recognize fetal antigens, but their activation is tightly restrained by local regulatory mechanisms. In mice, depletion of maternal Tregs unmasks Tconv-mediated fetal rejection, demonstrating that tolerance is an active process dependent on Tregs (33). In humans, fetus-specific $CD4^+CD25^{bright}$ Tregs are selectively enriched in the decidua with concurrent suppression of local effector responses, showing that Tconvs are actively regulated rather than absent (34, 35).

Decidual $CD8^+$ T cells display a unique mixed signature of activation and dysfunction, expressing effector molecules such as IFN-γ, TNF-α, perforin, and granzymes, while simultaneously upregulating inhibitory receptors including PD-1, CTLA-4, and LAG-3 (36). Additional regulatory pathways mediated by HLA-G and Indoleamine 2,3-dioxygenase

(IDO) restrain cytotoxicity under steady-state conditions, yet permit reactivation during infection, enabling T cells to tolerate fetal alloantigens like paternal HLA-C while still mounting antiviral responses (37).

Tregs can arise through two developmental routes: thymus-derived Tregs (tTregs) and peripherally induced Tregs (pTregs). Both lineages contribute to maternal-fetal tolerance. In mice, extrathymic induction of pTregs during pregnancy requires the FOXP3 CNS1 enhancer, and CNS1 deficiency impairs pTreg generation, disrupts spiral artery remodeling, and leads to increased fetal resorption, demonstrating that pregnancy-specific pTreg induction is crucial for placental morphogenesis (38). Another study in mice showed that Treg depletion during early placental morphogenesis caused impaired remodeling of decidual spiral arteries and fewer and dysfunctional uterine NK cells, resulting in fetal loss and fetal growth restriction (39). In humans, detailed phenotyping has revealed a multilayered decidual Treg compartment, consisting of three distinct $CD4^+$ Treg subsets: $CD25^{HI}FOXP3^+$, $PD1^{HI}IL\text{-}10^+$, and $TIGIT^+FOXP3^{dim}$ Tregs (35). Each subset has specialized suppressive programs that converge to restrain local effector responses. Decidual $PD1^{HI}$ Tregs suppress T cell proliferation in an IL-10-dependent manner (35). $CCR8^+$ decidual Tregs in mice, displaying a thymic-derived phenotype, display particularly strong suppressive capacity and are indispensable for maternal-fetal tolerance, and this subset of Tregs was found to be decreased at the decidua in women with RPL (40). Additional studies confirm that fetal antigen-driven expansion of maternal Tregs occurs and that these antigen-specific cells persist throughout gestation (41, 42).

The suppressive function of Tregs is mediated not only by their abundance and antigen specificity, but also by their expression of co-inhibitory molecules. Decidual Tregs express CTLA-4, which plays a central role in their ability to suppress local T cell activation (33, 35) In addition, trophoblast cells contribute to shaping the tolerogenic environment: expression of PD-L1 provides inhibitory signaling via PD-1 on maternal T cells, while HLA-G, a non-classical HLA class I molecule expressed by EVT, engages inhibitory receptors such as LILRB1 on maternal leukocytes (2). Taken together, maternal T cell responses are restrained to protect the semi-allogeneic fetus while allowing placental development to proceed.

Dendritic cells and macrophages

Dendritic cells (DCs) and macrophages are pivotal regulators at the maternal-fetal interface, where they coordinate tolerance and placental development. Their phenotypes and activities dynamically shift in response to local signals and are linked to pregnancy health and outcomes. The majority of the decidual macrophage population has an M2 phenotype, in that most of the cells express scavenger receptors CD163 and CD206, and that they show high phenotypic resemblance to cultured macrophages that are stimulated with M-CSF and

IL-10, by producing IL-10, IL-6, TNF, and CCL4 (43, 44). However, macrophage subtypes can be further distinguished based on CD11c and DC-SIGN (CD209) expression (43). Decidual macrophages secrete VEGF and enzymes including matrix metalloproteinases (MMPs), which mediate trophoblast invasion and spiral arterial remodeling (45). Macrophages contribute to the arterial remodeling by clearing extracellular matrix debris and phagocytose apoptotic vascular smooth muscle cells, while Wnt5a and IL-33 expression help the invasion of EVTs (43). The dysregulation of macrophage polarization is associated with a variety of pregnancy complications, including RPL, preeclampsia, fetal growth restriction and preterm labor (46).

In mice, it was found that antigen presentation by maternal APCs commenced only at mid-gestation when trophoblast debris was sampled and therefore maternal T cells recognized fetal antigens through indirect presentation (47). This delayed and restricted presentation may contribute to maternal T-cell "ignorance" of the fetus and prevents cytotoxic effector programming (47). As main APCs, the DC populations within the decidua undergo dynamic changes across gestation. In mice, uterine DCs are indispensable for implantation and decidualization, as their ablation disrupts decidual angiogenesis and proliferation, leading to embryo resorption even in syngeneic pregnancies (48). Beyond their role in antigen presentation, uterine DCs fine-tune angiogenesis by secreting soluble factors sFlt1 and TGF-β1, which regulate vascular permeability and maturation. Murine uterine $CD11c^+$ DC numbers increase after implantation, coinciding with a shift from a pro-inflammatory to a tolerogenic cytokine profile (49). In murine uterine-draining lymph nodes, the balance between conventional DCs and plasmacytoid DCs is critical, with an expansion of plasmacytoid DCs supporting Treg induction, while inflammatory disruption of this balance impairs tolerance and leads to fetal loss (50).

In parallel, placental macrophages of fetal origin, called Hofbauer cells (HBCs), emerge from placental erythro-myeloid progenitors and exhibit a primitive phenotype early in gestation (51). These macrophages are characterized by epigenetic silencing of HLA-DR, reflecting limited antigen-presenting capacity, and show transcriptional similarity to yolk-sac-derived macrophages (52). Despite their restricted antigen presentation, HBCs secrete a repertoire of cytokines and proteases, including IL-8 and MMP-9, that contribute to angiogenesis, extracellular matrix remodeling, and trophoblast invasion, while also increasing antimicrobial responses upon Toll-like receptor stimulation (52). Progressive acquisition of HLA-DR expression later in gestation suggests developmental tuning of macrophage function in parallel with placental maturation (51).

Allorecognition of the trophoblast and its immune regulating mechanisms

HLA molecules

Trophoblasts exhibit diverse immune-modulating properties. One of the mechanisms that trophoblasts use to escape and modulate the maternal immune response is a selective expression pattern of HLA (10). HLA is part of the Major Histocompatibility Complex (MHC) on chromosome 6 and is essential for the immune system to recognize and respond to pathogens (53). These highly variable glycoproteins are divided into class I and class II. HLA class I molecules include the classical polymorphic HLA-A, -B and -C. Classical HLA class I molecules are present on almost all nucleated cells and present peptides from inside the cell to $CD8^+$ T cells, which then target and kill infected or abnormal cells. HLA class II molecules consist of HLA-DR, -DQ, and -DP. These molecules are found on specialized immune cells and present peptides from outside the cell to $CD4^+$ T cells, which help coordinate the immune response. The non-classical HLA molecules are HLA-E, -F, and -G and are far less polymorphic. HLA-E regulates NK function through the NKG2A/CD94 receptor and presents conserved leader sequence peptides from other HLA class I molecules (54). HLA-F is the least studied HLA molecule and its function is not well defined (55). It may present long peptides or act as a chaperone for other class I molecules, including peptide free HLA. HLA-F can interact with inhibitory as activating NK cell receptors such as ILT2, KIR3DS1 and KIR3DL2. HLA-G is a strongly immunosuppressive molecule that exists in both membrane-bound and soluble forms and can inhibit NK cells, T cells, B cells, and myeloid cells through receptors such as KIR2DL4, ILT2, and ILT4 (56). T cells recognize antigens only when they are presented by HLA molecules, a process crucial for defending against infections, cancer, and involved in causing autoimmune damage and rejection of a transplanted organ.

HLA is co-dominantly expressed and inherited as haplotypes, with one set of genes from each parent. At the population-level, the diversity in HLA alleles is enormous. This diversity, polymorphism, resides in the antigen binding groove of the HLA molecules and serves to permit binding of a wide variety of (foreign) peptides. It serves to helps protect the population as a whole against widespread vulnerability to new or evolving pathogens. During pregnancy, this genetic diversity poses a unique challenge. The fetus is semi-allogeneic, carrying paternal HLA alleles that can potentially be recognized by the maternal immune system. Maternal T and B cells may respond to these foreign antigens, either indirectly through peptide presentation or directly to paternal HLA, potentially leading to pregnancy complications. However, this is mitigated partly by the fact that fetal trophoblast cells do not express HLA-A and HLA-B or any class II molecules (10). Instead, EVT only express a distinct repertoire of HLA molecules, limited to HLA class-I molecules; HLA-C, HLA-E, HLA-F and HLA-G, while STB lack HLA expression entirely. Notably, all fetal HLA antigens

can still be indirectly presented to maternal immune cells by maternal HLA molecules on (professional) antigen presenting immune cells such as DCs and macrophages.

Importantly, the exact mechanisms of fetal antigen recognition are not yet fully elucidated (2). Because STBs lack HLA expression, they are invisible to systemic HLA-restricted T cells, and the absence of HLA class II on trophoblasts prevents direct $CD4^+$ T cell recognition. Indirect recognition by $CD4^+$ T cells is theoretically possible via HLA class II-expressing decidual APCs, but it would require migration to lymph nodes and back, a process that is poorly understood in humans. $CD8^+$ T cell responses are restricted to HLA-C as the only classical HLA class I molecules on EVT, yet evidence for such responses in early human decidua is lacking. Key unknowns and controversies include whether the human decidua has functional lymphatic drainage, whether paternal HLA-C-restricted T cells are generated early, and how tolerogenic features of the decidua influence potential allorecognition. In this context, maternal-fetal HLA compatibility is particularly important to study, as (mis) matches in HLA may directly influence placentation processes and ultimately pregnancy outcomes.

HLA-C is of particular interest as the only polymorphic classical HLA class I molecule expressed by EVT. As described in section 1.3, the combination of maternal KIR genotype and fetal HLA-C allotype influences dNK cell function during placentation and is associated with pregnancy outcome.

HLA-G interacts with LILRB1 and KIR2DL4 receptors on NK and T cells, inducing immunosuppresion through inhibition of cytotoxicity and cytokine release (56). Beyond direct inhibition, HLA-G on EVT also promotes maternal tolerance by influencing the induction and persistence of Tregs (42). HLA-G also promotes NK cell activity contributing to trophoblast invasion and spiral artery remodeling as soluble HLA-G binding to KIR2DL4 and LILRB1 activates the ERK signaling pathway and increases production of tissue remodeling-associated proteases and proangiogenic cytokines and chemokines (56).

Immune-modulating factors, co-inhibitory molecules and inflammatory pathways
In addition to HLA molecules, trophoblasts express a range of immune-modulating factors. Trophoblasts secrete IL-10, a potent anti-inflammatory cytokine that in turn enhances HLA-G expression, promotes an M2 macrophage phenotype and downregulates co-stimulatory molecules (58-60). Likewise, transforming growth factor-beta (TGF-β) is a multifunctional cytokine that plays a key role in immune homeostasis and promotes fetal tolerance by regulating the maternal T cells, NK cells and macrophages (61, 62). It facilitates trophoblast invasion, spiral artery remodeling, and placental growth via SMAD-dependent and independent pathways (61, 62). TGF-β also contributes to expanding regulatory T

cells and dampening inflammatory immune responses at the decidua (61, 62). Lastly, hormone secretion, mainly by STB, plays an important role in mediating T cell responses. Progesterone shifts maternal T cell cytokine profiles away from Th1 responses (pro-inflammatory subtype of $CD4^+$ T cells) as it reduces IFN-γ and TNF-α and promotes Th2 (anti-inflammatory subtype of $CD4^+$ T cells) cytokine production, notably IL-4 (63). Estrogen and β-subunit of human chorionic gonadotropin (β-hCG) also support Treg induction and migration (64).

The trophoblast also employs the expression of co-inhibitory checkpoint and related suppressive molecules. Programmed death-ligand 1 (PD-L1) is expressed by STB and EVT and binds to PD-1 on maternal T cells, thereby inhibiting their activation and cytokine production (19, 65). PD-L1 is also expressed on decidual DCs and macrophages, where it contributes to the polarization of macrophages toward an anti-inflammatory M2 phenotype and promotes Treg proliferation (66). IDO suppresses T cell proliferation by depleting tryptophan and promotes regulatory responses, an effect that is enhanced by co-inhibitory interactions such as CTLA-4 signaling (65, 66). CD200 (also known as OX-2) on trophoblasts interacts with its receptor CD200R on macrophages to inhibit pro-inflammatory signaling (66). The expression of Galectin-9 by trophoblasts promotes dNK function through its receptor TIM-3 (66). Additionally, trophoblasts express complement regulatory proteins, which protect against complement-mediated damage (67). Together, these molecules form a complex immunoregulatory network that promotes fetal tolerance.

In general, despite the mechanical and cellular disruptions associated with implantation and trophoblast invasion, classical inflammatory responses are largely absent in early human decidua (2). Key hallmarks of acute inflammation such as mast cells, neutrophil infiltration, granulation tissue, and typical wound-healing angiogenesis, are either absent or tightly restricted, and anti-inflammatory factors such as TGF-β, actively modulate the local environment (2, 68). Gene expression patterns previously described as “inflammatory” in the endometrium largely reflect physiological processes, including NK cell proliferation and immune regulation, rather than tissue-damaging inflammation (69). Inflammatory features observed in tissue after miscarriage represent secondary responses, not the baseline decidual environment. In parallel, inflammatory processes thought to prepare for labor may influence the interpretation of findings in term placenta. Pregnancy encompasses a constant interplay and moderation by local cytokines, immune cells, exosomes, and hormonal signals, with fine temporal and spatial control (70). Collectively, these observations suggest that successful implantation and placentation rely on a specialized, tightly regulated immune milieu, rather than classical inflammatory pathways, highlighting the unique immune adaptation of the maternal-fetal interface.

Preeclampsia: placental pathology and immune dysregulation

Clinical manifestation and pathophysiology

Hypertensive disorders of pregnancy remain a major cause of maternal and perinatal morbidity and mortality worldwide. These disorders include pregnancy-induced hypertension (PIH) and preeclampsia (71). Preeclampsia is a multisystem pregnancy-specific disorder affecting approximately 3-5% of pregnancies, characterized by hypertension in combination with one or more of the following: proteinuria, other maternal organ dysfunction (e.g. renal insufficiency, liver involvement, neurological complications or hematological complications), or uteroplacental dysfunction manifested as fetal growth restriction or intrauterine fetal death (72). Although proteinuria was once required for diagnosis, definitions were revised in 2014 and updated most recently in 2021 (71, 73). The classification of preeclampsia remains under discussion, and can be based on severity (mild to severe and considering e.g. with or without HELLP syndrome (hemolysis, elevated liver enzymes and low platelets)), gestational age at clinical presentation (pre-term, term or postpartum), and early or late onset (74). Increasing scientific evidence supports a mechanistic classification, distinguishing subtypes based on underlying pathophysiology, such as angiogenic imbalance, maternal cardiovascular dysfunction, or immune maladaptation, which may offer more predictive and therapeutic relevance (75, 76).

While many cases follow a mild course, preeclampsia can lead to severe complications such as eclampsia, placental abruption, or hemorrhage if not properly managed (72, 74). It remains a leading cause of adverse maternal and neonatal outcomes globally (77). Beyond pregnancy, women affected by preeclampsia are at higher risk of developing long-term cardiovascular, renal, and neurological disease, along with reduced quality of life and reduced life expectancy (78). Meanwhile, babies from preeclamptic pregnancy have increased risks of preterm birth, perinatal death and neurodevelopmental disability and cardiovascular and metabolic disease later in life. To date, no curative treatment exists except for delivery of the fetus and placenta. Current management focuses on early identification of high-risk individuals, prophylactic low-dose aspirin, timely diagnosis, symptom control, and optimal timing of delivery (71).

Although symptoms generally occur in the third trimester, the pathogenesis of preeclampsia likely originates early in pregnancy, during the establishment of the placenta (74). As described above in section 1.2, normal placentation involves EVT invasion into the uterine wall, transforming spiral arteries into high-capacitance, low-resistance vessels that ensure adequate fetal perfusion. In preeclampsia, this spiral remodeling process is inadequate, resulting in placental hypoxia and oxidative stress (79, 80). These processes involve increased levels of anti-angiogenic factors such as soluble fms-like tyrosine

kinase-1 (sFlt-1) and soluble endoglin in maternal blood. Numerous mediators contribute to a systemic response, including pro-inflammatory cytokines, extracellular vesicles, and anti-angiogenic factors that impair vascular endothelial growth factor (VEGF) and suppress pro-angiogenic placental growth factor (PlGF) signaling. These factors promote maternal endothelial dysfunction and systemic multiorgan disorder, which involves reduced vasodilation, systemic inflammation, and thrombosis resulting in hypertension, liver and renal impairment, thrombocytopenia, and coagulopathy. The pathophysiology is therefore described as a two-stage model, with placental dysfunction causing STB stress as stage 1, and systemic endothelial dysfunction later in pregnancy leading to the clinical manifestation as stage 2 (81).

Immune dysregulation

Immunological factors are thought to play a central role in the development of preeclampsia. As described above, during implantation, EVT invade the decidua and interact with maternal immune cells and therefore encounter the paternal antigens of the semi-allogeneic fetus. Several epidemiological risk factors for preeclampsia, including first pregnancy, new paternity in multiparous women, prolonged intervals between pregnancies, use of donor oocyte or sperm, and barrier contraception, support the notion that insufficient immune adaptation contribute to its development (74). Repeated exposure to paternal antigens through seminal fluid appears protective, likely via the expansion of paternal antigen-specific memory Tregs (8). In preeclampsia, a reduction in both the number and function of maternal Tregs has been consistently observed, indicating a breakdown in immune tolerance mechanisms (82). More specifically, Th1 and Th17 (pro-inflammatory subtypes of $CD4^+$ T cells), disrupt immunological tolerance, whereas $CD4^+CD25^+Foxp3^+$ T reg cells promote immune tolerance (82, 83). Experimental studies in rat models of reduced uterine perfusion pressure (RUPP) have shown that infusion of Th17 cells induces hypertension and fetal growth restriction, while transfer of Tregs from normal pregnancies reduces hypertension and improves uteroplacental perfusion (84, 85). Early depletion of Tregs leads to placental hypoplasia and elevated uterine artery resistance, underscoring their role in vascular regulation and placentation. Subsequent increase of soluble endoglin neutralizes TGF-β, further inhibiting the differentiation into Treg cells and promoting differentiation into inflammatory Th17 cells (86). In humans, circulating and decidual Treg numbers are reduced in preeclampsia, alongside their impaired induction by $CD14^+$DC-SIGN$^+$ APCs and increased apoptosis, suggesting both defective differentiation and enhanced cell death contribute to Treg depletion (82). Tregs can be further classified into paternal/fetal antigen-specific and nonspecific subsets; the former expand clonally in response to fetal antigens, persist after parturition, and rapidly increase in subsequent pregnancies, likely promoting tolerance and successful placentation. In preeclampsia, the proportion of clonally expanded Tregs in the decidua is markedly reduced, while an increase of clonal PD-1$^-$$CD8^+$ T cells is observed

(82, 87). Studies on the role of the PD-1/PD-L1 axis in preeclampsia remain contradictory, as human peripheral level of PD-1 and placental PD-L1 are reported to be reduced, while PD-L1 blockade in rats has a protective effect through enhanced Treg activity (19, 66, 87)

As previously discussed in section 1.3, specific maternal KIR and fetal HLA-C interactions are associated with aberrant dNK cell responses that may lead to shallow EVT invasion and impaired spiral artery remodeling (31). Specifically, the combination of maternal KIR AA genotype and fetal HLA-C2 is associated with an increased risk of preeclampsia (28, 29). In this case, the HLA-C2 on EVT is expected to only engage the inhibitory KIR2DL1 receptor, causing strong inhibition of dNK cell function.

Immunological dysregulation underlying preeclampsia is further demonstrated by a variety of studies that found HLA-G gene polymorphisms and decreased levels of sHLA-G to be related to the incidence of preeclampsia (56). Other immune-related disruptions in preeclampsia include an imbalance of M1 and M2 macrophages or by a reversal to M1 macrophages, dysfunctional DCs and enhanced complement activation (88). These features may lead to a loss of inhibition of local immune cells, promoting inflammation and impairing trophoblast invasion. Together, these observations underscore the central role of immune dysregulation in the pathogenesis of preeclampsia. Clarifying the specific mechanisms of maternal-fetal immune interactions is essential for improving prediction and guiding the development of preventive or therapeutic strategies.

Oocyte donation pregnancies: high immunogenic dissimilarity at risk of preeclampsia

Oocyte donation (OD) is of particular interest in this dissertation for two key reasons: first, because it represents a high-risk pregnancy with a significantly increased risk of hypertensive complications, including preeclampsia; and second, because it provides a unique model to investigate maternal-fetal immune interactions. OD is an increasingly applied assisted reproductive technique (ART) that enables women without functional oocytes, or those carrying genetic risks, to achieve pregnancy. In OD, an oocyte from a related or unrelated donor is fertilized by sperm from the intended father and transferred into a hormonally prepared recipient uterus. The number of OD cycles has risen steadily over the past decades, encompassing 8-18% of all ART cycles, which is a trend expected to rise even further (89-91). In the Netherlands, OD is legally permitted only on a non-commercial, non-anonymous basis, with premature ovarian failure as the most common indication (92, 93). Additional indications include diminished ovarian reserve, advanced maternal age, IVF failure, reception of oocytes from partner (ROPA), and genetic concerns. Due to legal and logistical limitations, many Dutch couples travel abroad for OD treatments, where commercial and anonymous donation is allowed and multiple embryo transfers are

more common. This cross-border care increases the risk of multiple gestations, which are associated with higher maternal and fetal complication rates. Despite high reproductive success, OD pregnancies carry an increased risk of pregnancy-induced hypertension, preeclampsia, preterm birth, growth restriction, low birth weight, cesarean section, postpartum hemorrhage, and first-trimester bleeding compared to natural conception and autologous IVF/ICSI (94-100).

OD pregnancies offer a unique model for studying maternal-fetal immune interactions, as the fetus is fully allogeneic to the gestational carrier. This immunogenetic disparity, thus related to a high level of maternal-fetal HLA mismatches, resembles aspects of solid organ transplantation, where donor antigens can trigger rejection. Although acute rejection, driven by direct recognition of donor HLA, is unlikely in pregnancy due to the absence of fetal antigen-presenting cells in maternal tissue, indirect antigen presentation may still occur. Fetal microparticles present in maternal blood can be processed by maternal dendritic cells and presented via HLA class II molecules to $CD4^+$ T cells, potentially contributing to local immune activation. Interestingly, successful OD pregnancies show more HLA matching than expected by chance, suggesting a possible role for partial HLA compatibility in modulating maternal tolerance (101). Uncomplicated OD pregnancies also show features of increased compensation for high allogenicity, such as higher Treg frequency at the decidua basalis and immunoregulatory cytokines in maternal peripheral blood, both related to the level of HLA mismatches (102, 103). Studies have shown that placentas of OD pregnancies more frequently exhibit inflammatory histological lesions that include chronic deciduitis and villitis of unknown etiology, and display impaired spiral artery remodeling (104-106). Furthermore, increased numbers of immune cells in the decidua have been documented, both associated with beneficial and adverse pregnancy outcomes. (104, 107-109).

The significantly higher incidence of preeclampsia in OD pregnancies underscores the role of immune dysregulation in its pathogenesis. Because OD represents the most extreme form of fetal allogenicity, it offers a naturally occurring model to study how maternal immune tolerance is challenged and may fail in some cases. The combination of clinical vulnerability and immunogenetic disparity makes OD a unique opportunity to investigate the immunological mechanisms that underlie preeclampsia, and more broadly, to gain insight into the limits and adaptability of maternal-fetal immune interactions.

Aim and outline of this dissertation

The immune system plays a dual role in pregnancy: it must maintain tolerance toward the semi-allogeneic fetus, while protecting the mother and fetus from infections. Understanding the immunological mechanisms that enable this balance is key to unraveling the pathogenesis of immune-mediated complications such as preeclampsia. This

dissertation aims to explore the pathways by which maternal-fetal tolerance is established and how these are disrupted in preeclampsia, with a particular focus on maternal-fetal HLA compatibility and trophoblast-mediated modulation of maternal $CD4^+$ T cell responses.

Chapter 2 investigates the role of and potential selection for maternal-fetal HLA compatibility in preeclamptic and uncomplicated pregnancies. The study assesses the frequency and nature of HLA matches and mismatches and explores maternal KIR-fetal HLA-C interactions in relation to pregnancy outcome. This chapter demonstrates the link between maternal-fetal HLA compatibility and maternal immune (dis)regulation.

Chapter 3 focusses on a genetically isolated population to determine how low HLA diversity influences maternal-fetal HLA compatibility and the predicted strength of $CD4^+$ T cell responses to fetal antigens in relation to PIH and preeclampsia. This chapter further underlines the contribution of maternal-fetal HLA mismatches to immune regulation, pointing to an immunogenetic basis of hypertensive pregnancy disorders.

Chapter 4 evaluates the feasibility of isolating EVT from maternal cervical samples in early pregnancy for the purpose of non-invasive fetal HLA typing. The technical optimization and potential applications of this method are discussed in the context of early risk stratification for preeclampsia. This approach holds promise for developing minimally invasive tools for fetal genomic screening to identify pregnancies at increased risk.

Chapter 5 examines the expression of key immunomodulatory molecules including PD-L1, CD200, and IDO, in the term placenta of OD pregnancies compared to naturally conceived pregnancies, investigating both uncomplicated and preeclamptic cases. These findings advance our understanding of how altered immune modulation in OD pregnancies may predispose to preeclampsia.

Chapter 6 presents an in vitro co-culture model in which primary EVT from first-trimester placentas are cultured with maternal conventional $CD4^+$ T cells to study the potential of these trophoblasts to induce regulatory T cell features. This study provides mechanistic insight into how trophoblasts may shape maternal T cell responses to support tolerance in early pregnancy.

Collectively, this dissertation aims to enhance our understanding of maternal-fetal immune interactions and to identify immunological factors that may serve as future targets for the prediction, prevention, or treatment of preeclampsia.

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