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Multi-Layered Mechanisms of Immunological Tolerance at the Maternal-Fetal Interface

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ABSTRACT

Pregnancy represents an immunological paradox where the maternal immune system must tolerate the semi-allogeneic fetus expressing paternally-derived Ags. Accumulating evidence over decades has revealed that successful pregnancy requires the active development of robust immune tolerance mechanisms. This review outlines the multi-layered processes that establish fetomaternal tolerance, including the physical barrier of the placenta, restricted chemokine-mediated leukocyte trafficking, lack of sufficient alloantigen presentation, the presence of immunosuppressive regulatory T cells and tolerogenic decidual natural killer cells, expression of immune checkpoint molecules, specific glycosylation patterns conferring immune evasion, and unique metabolic/hormonal modulations. Interestingly, many of the strategies that enable fetal tolerance parallel those employed by cancer cells to promote angiogenesis, invasion, and immune escape. As such, further elucidating the mechanistic underpinnings of fetal-maternal tolerance may reciprocally provide insights into developing novel cancer immunotherapies as well as understanding the pathogenesis of gestational complications linked to dysregulated tolerance processes.

Keywords: Pregnancy; Immune tolerance; Maternal-fetal interface

INTRODUCTION

The immune system is a specialized defense system that protects our body from invading foreign molecules and pathogens. In particular, B cells and T cells acquire the ability to distinguish self from non-self Ags through selection procedures during lymphocyte development. Thus, early studies suggested that pregnancy is an immunological paradox since the fetus of a pregnant mother carries semi-allogeneic Ags derived from paternally transmitted genes, and it would be conceivable for the maternal immune system to attack this allogeneic fetus (1). However, this catastrophic event does not occur in a normal pregnancy. Peter Medawar, who was interested in this subject, made a unique discovery and received the Nobel Prize for his work. Highly influenced by Macfarlane Burnet's theory and the work of Ray D. Owen, Medawar and his colleagues performed tissue graft experiments in a mouse model. They inoculated cells from one strain of mouse into a developing fetus of a different

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Abbreviations

APC, Ag-presenting cell; CIITA, class II transactivator; CNS1, conserved noncoding sequence 1; DC, dendritic cell; dNK, decidual NK cell; DSC, decidual stromal cell; ELF3, E74 like ETS transcription factor 3; EVT, extravillous trophoblast; hCG, human chorionic gonadotrophin; IDO, indoleamine 2,3-dioxygenase; ILT, Ig-like transcripts; KIR, killer cell Ig-like receptor; LAG3, lymphocyte activation gene 3; MMP, matrix metalloproteinase; NLRC5, NOD-like receptor family CARD domain containing 5; OVA, ovalbumin; PE, pre-eclampsia; pNK, peripheral NK cell; Rh, rhesus; RPL, recurrent pregnancy loss; SCT, syncytiotrophoblast.

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strain. Remarkably, they found that skin grafts from the first strain could then be accepted by the second strain, even in adulthood, when they would have been typically rejected (2). This phenomenon was termed immunological tolerance by Medawar and Burnet, who shared the Nobel Prize in 1960. While Medawar's work did not directly explain the mechanisms governing maternal tolerance of the semi-allogeneic fetus, it revealed the principle of acquired immunological tolerance for the first time.

REQUIREMENT OF ACTIVE IMMUNE TOLERANCE DEVELOPMENT FOR SUCCESSFUL PREGNANCY

With Burnet's clonal selection theory and Medawar's work, it was once thought that self-reactive clones were deleted *in utero*. However, later studies found that this is not the case, although the core principles of the clonal selection theory remain valid. Contrary to the initial postulation, it became evident that neither the uterus nor the fetus itself is intrinsically special; instead, active development of immune tolerance is required. For example, an early suggestion was that the maternal immune system remains inactive during pregnancy. However, this idea was negated by findings that fetal tissues transplanted into pregnant female rats were rejected as rapidly as organ allografts (3), demonstrating that fetal tissue itself does not possess unique properties for evading maternal immunity. Other studies showed that the uterus is not an immune-privileged site, as skin grafts syngeneic to the fetus were rejected when implanted in the uterus (4). In humans, immune reactivity toward fetal tissue is possible in rare cases. In rhesus (Rh) disease, Rh-negative mothers carrying Rh-positive fetuses develop Abs against the Rh Ag after exposure to Rh-positive blood cells. During subsequent pregnancies with Rh-positive fetuses, these Abs can cross the placenta and attack the fetus, leading to complications (5). Collectively, these reports underscore that active immune tolerance mechanisms are essential for successful pregnancy.

Now even more extensive experimental evidence has accumulated supporting the requirement for active immune tolerance development during pregnancy. Studies in rodent models clearly demonstrate that fetal-reactive immunity can arise but is actively suppressed in normal pregnancy. Rare cases of high abortion rates during pregnancy between CBA females and DBA males originate from semi-allogeneic immune responses, leading to complement activation and dysregulated angiogenesis (6-8). In this abortion-prone mating combination, inhibiting CD80 and CD86 signaling prevents maternal rejection of the allogeneic fetus (9), suggesting the involvement of the adaptive immune system. This idea is markedly illustrated by models utilizing defined fetal Ags like ovalbumin (OVA) (10). When a female mouse is pregnant with an OVA-expressing fetus, the fetal Ag sheds into the maternal circulation (11), where it is captured by maternal Ag-presenting cells (APCs) and presented to T cells. However, the T cells recognizing this fetal alloantigen become inactivated to anergy state (10). In a setting where all maternal CD4+ T cells are specific for a fetal Ag, achieved by breeding OT-II females with OVA-expressing males, tolerance to the fetus is maintained (12). This is due to the deletion of fetal-Ag-specific CD4+ T cells, downregulation of the TCR, and upregulation of immune checkpoint molecules like PD-1 and CTLA-4 (12). Moreover, fetal-Ag-specific Tregs are increased in this setting (12). However, the tolerogenic effects are less effective in CD8+ T cells, as fetal loss is observed when OT-I females are bred with OVA-expressing males (12). Given the importance of Tregs in suppressing immune responses against fetal Ags (13), the lack of Tregs may permit the cytotoxic effects of CD8+ T cells toward fetal Ags in OT-I mice. Collectively, these findings indicate that fetal Ags can

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be present in the maternal system, underscoring the necessity of active immune tolerance development for successful pregnancy.

MECHANISMS OF IMMUNOLOGICAL TOLERANCE DEVELOPMENT DURING PREGNANCY

Studies have revealed multi-layered mechanisms that protect the semi-allogeneic fetus from maternal immune system (**Fig. 1**). Here we present the key players in this process, focusing on the placenta's barrier properties, immune evasion of trophoblasts by absence of Ag presentation, as well as the roles of various maternal immune cells, such as natural killer cells, Tregs, and additional molecular features that contribute to immune tolerance at maternal-fetal interface. We will also discuss how this knowledge could be translated into clinical applications.

Physical barrier

The fetus is separated from the mother by cellular barriers in the placenta, although the histological structure of the placenta varies across mammalian species. The human placenta is a hemochorial placenta, in which fetal trophoblasts are bathed in maternal blood (14). The outermost layer, the syncytiotrophoblast (SCT), functions as a cellular barrier and controls the exchange of substances between the fetus and mother (15). Despite the presence of a small number of fetal and maternal cells in each other's compartments, the placenta acts as a major barrier, limiting the transmission of semi-allogeneic fetal Ags to the mother or vice versa. Previous studies on transplacental cell passage have shown that only a small

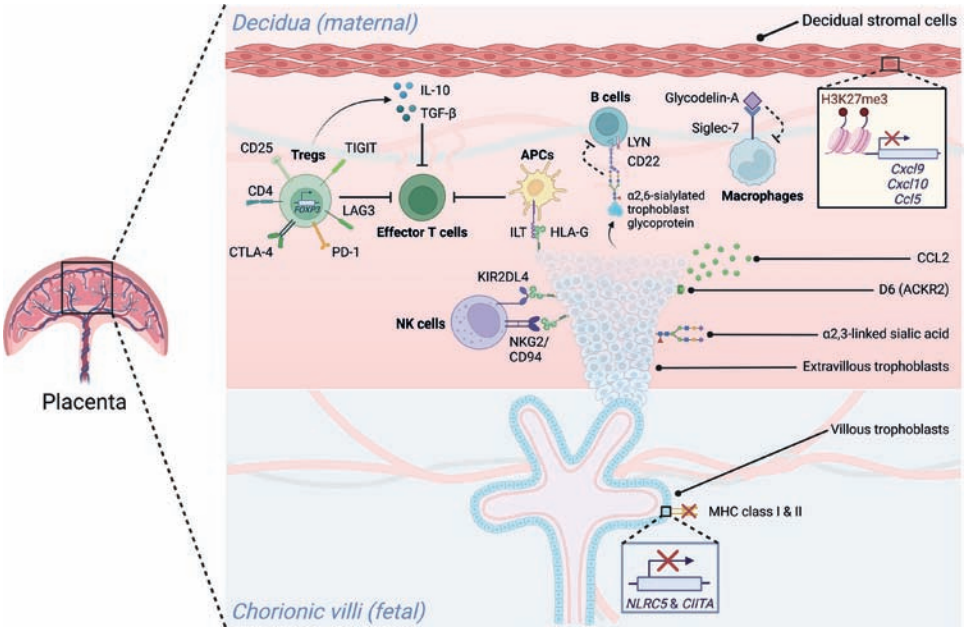

Figure 1. Overview of mechanisms of immunological tolerance at the maternal-fetal interface. While the semi-allogeneic fetal cells and antigens are continuously exposed to the mother, the immune system in maternal-fetal interface promotes a tolerogenic environment for the fetus. Placental barrier restricts the transfer of fetal antigen to maternal circulation. Maternal immune cells including dNKs, antigen-presenting cells, monocytes, and T cells exhibit regulatory phenotype during gestation. Additionally, deprivation of immune-activating molecules such as chemokines and MHC antigens protects the fetus from maternal immune attack. The glycosylation process in placenta also favors tolerance by suppressing the maternal immune system.

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percentage of cells may be transferred across the placenta (16-18). During gestation, maternal cells are found in fetal tissues such as the thymus, spleen, and liver (19). While the specific population of transferred cells was not identified, it appears that leukocyte transfer is more restricted than that of erythrocytes (18). Additionally, although maternal immune systems may be primed to fetal Ags, this is not a common occurrence in normal human pregnancies (20). Experiments in mice have further revealed that sensitization to fetal alloantigens can occur, but the fetus is not rejected due to inhibited access to the maternal immune system (21,22). These findings further underscore the pivotal role of the placental barrier in establishing maternal-fetal immune tolerance.

The placenta also functions as an immunoabsorbent for maternal Abs specific to fetal Ags (23). This phenomenon is not dependent on the binding of paternal Ag-specific Abs to other maternal organs or Fc receptor-mediated binding of Abs in the placenta but is instead dependent on the antigenic specificity of the F(ab')2 Ab fragments (23,24). Thus, the placenta serves as a barrier that not only passively restricts contact between the fetus and mother but also actively blocks maternal immune attacks against the fetus.

Immune barrier: chemokines

During mammalian pregnancy, maternal immune cells are recruited to the maternal-fetal interface and actively regulate specialized immune responses between mother and fetus. Additionally, the placenta expresses a wide range of chemokines and their receptors (25). However, activation of chemokine signaling may recruit and dysregulate maternal leukocytes and trigger inflammatory responses within the placenta. Thus, the proper regulation of chemokine and chemokine receptor expression appears to be necessary for a successful pregnancy.

One chemokine receptor expressed in the placenta with a protective role against placental inflammation is D6, also known as atypical chemokine receptor 2 (26-28). D6 is expressed in extravillous trophoblasts (EVTs) and on the apical side of SCTs, where it scavenges the inflammatory CCL2. Genetic ablation of D6 in pregnant mice results in increased levels of inflammatory chemokines and leukocyte infiltration into the placenta under inflammatory conditions (26). Moreover, the loss of D6 is associated with structural defects in the placenta and an increased incidence of adverse pregnancy outcomes (27). Notably, trophoblastic D6 prevents CCL2 from entering the embryonic circulation, suggesting the 'compartmentalization' of maternal chemokines within the decidua (28). Interestingly, Nancy et al. (29) demonstrated that chemokine gene silencing in decidual stromal cells (DSCs) inhibits the infiltration of T cells into the decidua. The chemokine expression in DSCs was epigenetically repressed by H3 trimethyl lysine 27 histone on the promoters of gene *Cxcl9*, *Cxcl10*, and *Ccl5*. The ectopic expression of CXCL9 and CCL5 in the decidua, leading to the infiltration of CD3+ T cells, further supports the notion that the lack of chemokine expression restricts T cell accumulation in this region (29).

Of note, aberrant regulation of placental chemokines is associated with adverse pregnancy outcomes (30,31). Clinical studies on pre-eclamptic women have revealed that the scavenging of CCL2 is reduced due to impaired D6 function in the placenta of these patients (30). Furthermore, the inflammatory chemokine CX3CL1, which is expressed by SCTs, is increased in the placenta of patients with pre-eclampsia (PE), and its expression level positively correlates with clinical and pathological parameters of PE (31).

Taken together, these findings highlight the critical importance of tightly regulating chemokine expression and function at the maternal-fetal interface to maintain an immune-privileged environment during pregnancy. Further studies are needed to investigate the regulation of additional chemokines and their receptors at the maternal-fetal interface.

Insufficient alloantigen presentation

MHC molecules present peptides on the cell surface to initiate adaptive immune responses. MHC class I molecules are expressed by all nucleated cells and present Ags of cytosolic origin, whereas MHC class II molecules are found primarily on specialized APCs and present exogenously derived Ags (32). Notably, in addition to their Ag-presenting capacity, MHC molecules function as determinants of immunological self and non-self. These MHC molecules are recognized by the adaptive immune system and allogenic cells expressing non-self MHC molecules are rejected (33). Since the fetus possesses semi-allogeneic MHC and antigenic peptides that may stimulate the maternal immune system, restricting alloantigen presentation is essential for maintaining fetomaternal tolerance.

Among MHC class I molecules, non-classical HLA-G Ag is specifically expressed by trophoblasts during gestation (34,35). In fact, trophoblasts are devoid of most of MHC Ags but EVTs which invade maternal decidual tissue and encounter maternal immune cells express high levels of HLA-G (35). HLA-G in trophoblasts is present as a homodimer that preferentially binds Ig-like transcripts (ILTs) on decidual APCs (36). This interaction induces a regulatory phenotype in decidual myelomonocytic cells, suppressing T cell responses and impairing Ag presentation via MHC class II molecules (37). NK cells also express other inhibitory receptors for HLA-G Ag such as killer cell Ig-like receptor (KIR) 2DL4 (38). Thus, the specific expression of HLA-G with the absence of classical MHC class I molecules, HLA-A and HLA-B in EVTs promotes tolerogenic microenvironment in maternal-fetal interface. Alongside HLA-G expression, EVTs express other MHC class I molecules HLA-C and HLA-E. Unlike HLA-A and HLA-B, which are major TCR ligands, HLA-C in EVTs does not activate maternal T cells but binds KIRs expressed on uterine NK cells, thereby promoting EVT function (39,40). Furthermore, while expression of MHC class I proteins is regulated by NOD-like receptor family CARD domain containing 5 (NLRC5), the master regulator of MHC class I molecules, expression of HLA-C is regulated by transcription factor E74 like ETS transcription factor 3 (ELF3) independently of NLRC5 (41). Expanding on these findings to clinical relevance, the expression of HLA-G in placenta and its release into maternal circulation is impaired in PE patients while expression of HLA-B is significantly elevated in these patients (42,43).

The absence of MHC class II Ag expression is another unique feature of placental immunity (44-46). It was initially assumed that trophoblasts failed to express class II transactivator (CIITA) gene due to methylation of IFN-γ inducible promoter of CIITA (45). However, Holtz et al. (46) reported that CIITA promoter is silenced by epigenetic mechanisms rather than promoter methylation. Considering that trophoblasts invade decidua and directly encounter maternal immune cells, it is not surprising that induction of MHC class II Ag in trophoblasts leads to fetal rejection by maternal immune attack (47). Furthermore, maternal uterine immune cells exhibit decreased expression of MHC class II molecules during gestation. Seminal fluid primes uterine CD11c+ cells to tolerogenic phenotype, thereby reducing MHC class II Ag expression and inducing expansion of paternal Ag-specific Tregs (48). MHC class II-expressing uterine dendritic cells (DCs) are also sequestered within the decidua during pregnancy to prevent active Ag transport and presentation to maternal T cells (49). Moreover,

pregnancy-related hormones such as human chorionic gonadotrophin (hCG) and TGF-β1 also reduce expression of MHC class II Ags and Ag presentation in uterine innate lymphoid cells. These results further demonstrate that MHC class II-dependent Ag presentation in the fetomaternal interface is highly suppressed (50).

In summary, the function of MHC class I and II molecules in the placenta is insufficient for alloantigen presentation. Consequently, defective Ag presentation systems in the placenta enable semi-allogeneic trophoblasts to invade maternal tissue effectively while safeguarding the fetus from potential cytotoxic responses by the maternal immune system.

NK cells

NK cells constitute the largest population of immune cells in the decidua, particularly during pregnancy, and play a critical role in remodeling and maintaining immune tolerance at maternal-fetal interface (51). While NK cells in peripheral tissues primarily exhibit cytotoxic functions, decidual NK cells (dNK cells) play a distinct role in immune suppression during gestation. The differentiation of dNK cells is tightly regulated to prevent tissue damage and promote remodeling of the spiral arteries, a process necessary for proper placental function (52). dNK cells are significantly distinct from peripheral NK cells (pNK cells) in their gene expression profiles and expression of immunosuppressive molecules such as KIR2DL4 and NKG2 receptors (53,54). For example, $CD56^{high}$ CD27+ dNK cells express substantially higher levels of inhibitory receptors compared to their CD27+ pNK cell counterparts (53). In mouse models of dNK cell differentiation, transcription factor *Eomes* exhibited preferential activation over *T-bet*. *T-bet* is crucial to the maintenance of pNK cell numbers and functions but exerts minimal effects on morphology of dNK cells and spiral artery remodeling (55).

dNK cells also recognize MHC class I molecules expressed on EVTs via their inhibitory receptors. Interactions between these MHC class I molecules and inhibitory receptors on dNK cells suppress immune responses by signaling through classical and non-classical HLA molecules. The CD94/NKG2 complex expressed on dNK cells binds to HLA-G and HLA-E expressed on EVTs, inducing functional inhibition of dNK cells and preventing cell lysis mediated by NK cells (54,56). Additionally, dNK cells express the inhibitory surface receptor KIR2DL4, which, upon binding to HLA-G, recruits the protein tyrosine phosphatase SHP-1 to immunoreceptor tyrosine-based inhibitory motifs, thereby inhibiting NK cell activation (57). ILT2, also known as leukocyte Ig-like receptor subfamily B member 1, is another HLA-G receptor expressed on the surface of dNK cells (58). ILT2 induces inhibitory signals upon engaging with HLA-G. Through the inhibitory receptors expressed on their surface, dNK cells prevent lysis of trophoblasts and suppress immune responses against the fetus. Genomic and epidemiological studies revealed that haplotype of maternal KIR and fetal HLA-C variants are associated with incidence of gestational complications such as pre-eclampsia and birthweights (59,60). Furthermore, dNK cells exhibit an immunosuppressive function by regulating the differentiation of Th17 cells. CD27+ dNK cells release high levels of IFN-γ, which suppresses Th17 cell development and function during pregnancy (61). These multifaceted mechanisms employed by dNK cells collectively create an immune-privileged environment at the maternal-fetal interface, safeguarding the semi-allogeneic fetus from maternal immune attack.

Tregs

Tregs represent another key cellular mediator of maternal-fetal tolerance, expanding systemically and locally during gestation to suppress immune responses against semi-

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allogeneic fetus through diverse molecular mechanisms. Expansion of CD4+ CD25+ Tregs during pregnancy can be alloantigen-specific (13). Fetal Ag-specific maternal Foxp3+ CD4 T cells are accumulated during pregnancy, persisted after parturition, and rapidly re-accumulated upon secondary pregnancy with the same paternal background (62). Moreover, alloantigen-independent expansion of Tregs has also been reported in other contexts (63). Aluvihare et al. (63) demonstrated that the maternal CD25+ T cell pool, which possesses potent regulatory functions, expands systemically during gestation, and the depletion of these cells leads to immunological rejection of the fetus . These pregnancy-induced Tregs are found to be generated extrathymically (64). Conserved noncoding sequence 1 (CNS1) is an enhancer region of the Foxp3 gene essential for extrathymic Treg differentiation but not for thymus-derived Tregs. Samstein et al. (64) found that paternal alloantigen-specific Tregs are generated in a CNS1-dependent manner, further suggesting that pregnancy-induced Tregs arise extrathymically.

Tregs also express immune checkpoint molecules, including CTLA-4, PD-1, and lymphocyte activation gene 3 (LAG3), on their surface (65). The expression and signaling via these molecules enhance the regulatory functions of Tregs. Interaction with their ligands and signaling through CTLA-4 controls expression of CD80/86 by APCs, PD-1 enhances the suppressive capacity of Tregs, and LAG3 negatively regulates T cell function (66-71). Studies have found that the expression of these immune checkpoint molecules is increased in Tregs during pregnancy (72,73). Moreover, Tregs release a variety of cytokines that modulate immune cells at the maternal-fetal interface. For example, the immune-suppressive cytokines IL-10 and TGF-β from Tregs play a central role in promoting immune suppression in the placenta during pregnancy (74,75). These cytokines diminish effector phenotype of T cells, inhibit activation and maturation of APCs, and further induce differentiation of T cells and monocytes into immunosuppressive Tregs and M2 macrophages (76,77).

Without proper development of Tregs, the decidua undergoes histological changes, including necrosis and thrombosis, leading to implantation failure and fetal loss (64). Consistently, the amount of Tregs is lower in women who have experienced recurrent spontaneous abortions compared to fertile women (78). Geng et al. (79) also showed that sphingosine-1-phosphate receptor 1, an inhibitor of Treg differentiation, is upregulated in placental T cells from patients with recurrent pregnancy loss (RPL). In contrast, intrauterine infusion of autologous Tregs in RPL patients alleviated miscarriage rates (80). Collectively, these findings show the critical role of Tregs in protecting the fetus against allogeneic immune responses.

Immune checkpoint molecules

Recent success of cancer immunotherapy has highlighted the importance of the immune checkpoint signaling in controlling immunity. Understanding the precise molecular mechanism of these signaling pathways has become one of the most important research subjects. However, the critical role of immune checkpoint molecules in establishing immune-suppressive environment at the maternal-fetal interface has been documented before the rise of cancer immunology (**Fig. 2**). For example, expression and regulation of immune checkpoint molecules such as CTLA-4, PD-1, and LAG3 have been reported in various T cells during pregnancy (72,74,81,82). Recent single-cell study provides an even more clear picture of this regulation. For instance, the expressions of checkpoint molecules such as PD-1, PD-L1, or TIGIT are identified among various immune cells and EVT, providing multiple layers of immune suppressive environment (83). Clinical evidence also supports the importance of immune checkpoint molecules that expression of PD-1 and population of PD-1+ immune

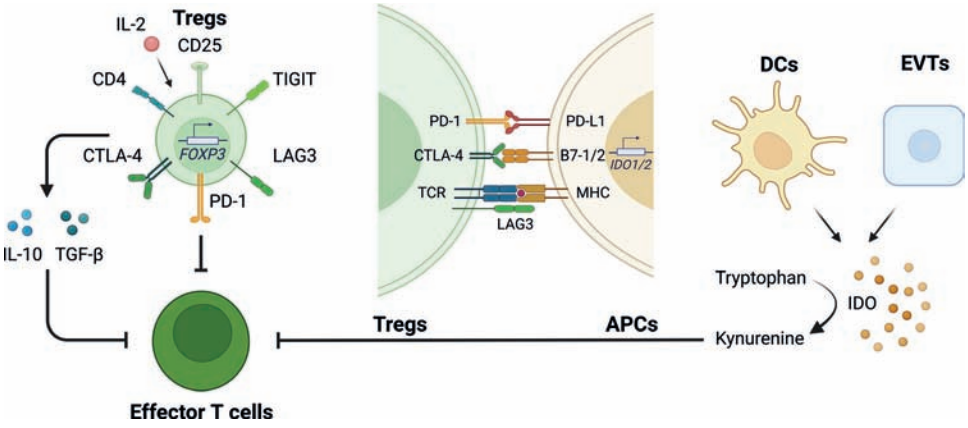

Figure 2. Markers of Tregs and immunosuppressive effects of immune checkpoint molecules. Tregs express molecular markers and immune checkpoint molecules that play immunosuppressive roles at the maternal-fetal interface. Additionally, IDO, expressed by DCs or EVTs, plays a crucial role in suppressing effector cells by catalyzing the conversion of tryptophan into kynurenine.

cells were significantly decreased in decidua of PE patients (84). In addition, patients with miscarriage exhibited lower levels of CTLA-4 expression in decidual and peripheral lymphocytes (85). Therefore, immune checkpoint molecules are also key factors in fetomaternal tolerance.

Glycosylation of molecules

There is also growing evidence that glycosylation, which is the most complex and diverse post-translational modification process, also contributes to immunological tolerance at maternal-fetal interface. First identified by Whyte and Loke, surface glycopeptides of trophoblasts are enriched with sialic acid and the authors postulated that sialylation may be associated with invasiveness of trophoblast itself (86). Later study confirmed that trophoblast subsets are differentially glycosylated as they exhibit different levels of invasiveness in placenta. Nonetheless, the most dominant form of sialylation in placenta was α2,3-linked terminal sialic acid, which is found at surface glycan arms of SCTs and cytotrophoblasts. Moreover, EVT only expressed α2,3-linked sialic acid residue in their N-glycans (87). Since then, many studies have focused on the biological role of sialylation during gestation.

Unexpectedly, lack of cell surface sialylation in trophoblast resulted in developmental failure by maternal immune attack. The immunologic rejection of sialic acid-deficient embryo was mainly mediated by maternal complement attack, which is rescued by exhaustion of complement factor C3 (88). Moreover, recent study by Rizzuto et al. (89) has shown that α2,6-linked sialic acid is also pivotal to the maintenance of immune tolerance. Heavily α2,6-sialylated trophoblast-derived Ags are recognized by specific receptor CD22 in maternal B cells. This interaction activated CD22-LYN signaling to suppress fetal Ag-specific maternal B cells and CD4+ T cells (89). Apart from glycosylation in trophoblasts, glycodelin-A is another sialylated glycoprotein in placenta. Among sialic acid-binding receptors, sialic acid-binding Ig-like lectin 7 expressed by monocytes binds decidual glycodelin-A and induces polarization of monocytes to regulatory decidual macrophage-like phenotype (90). Taken together, these results suggest that the sialylation process in placenta establishes a tolerogenic environment for a developing fetus. Further studies will be required to identify the versatile role of placental glycobiology in pregnancy.

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Other factors

Beyond cellular and molecular mechanisms of fetomaternal tolerance, metabolic pathways, and pregnancy-associated hormones also contribute critically to establishing an immunosuppressive milieu at the maternal-fetal interface, further highlighting the multifaceted nature of fetomaternal tolerance induction. One of the earliest studies highlighted the role of tryptophan metabolism at the maternal-fetal interface (**Fig. 2**). The enzyme indoleamine 2,3-dioxygenase (IDO), primarily produced by DCs, catalyzes the initial step of tryptophan metabolism by converting tryptophan to kynurenine (91). Kynurenine binding to the aryl hydrocarbon receptor on naive CD4+ T cells induces Foxp3 expression, thereby generating Tregs (92,93). Inhibition of tryptophan metabolism during pregnancy leads to fetal rejection mediated by T cell activation (94). IDO expression in DCs also suppresses T cell proliferation at least in part by the depletion of tryptophan in the microenvironment (91). Additionally, IDO is also expressed in giant trophoblasts in mice and EVT and villous trophoblasts in humans (95-97). Other studies report the significance of pregnancy-associated hormones on the immune-suppressive functions of immune cells and trophoblasts. For example, progesterone induces the differentiation of naive T cells toward Tregs and suppresses the differentiation toward Th17 cells in cord blood, which is associated with the enhancement of STAT5 and suppression of STAT3 (98). This process is crucial as Th17 cells have been implicated in various obstetric complications, such as RPL and preeclampsia (99). Progesterone also stimulates the expression of HLA-C and HLA-G in EVTs through non-classical progesterone receptor membrane component 1-ELF3 axis (100). hCG is one of the first messenger molecules from embryo to maternal cells during the process of implantation. The role of hCG in the immune-suppression is reviewed elsewhere (101). Due to the difference between humans and mice, mechanistic studies are still largely lacking to identify the exact role of hCG in immune regulation during pregnancy.

IMPLICATIONS AND TRANSLATIONAL PERSPECTIVES BEYOND PREGNANCY

Insights into the mechanistic pathways of fetal-maternal immune tolerance hold the potential for improving clinical outcomes in gestational complications. However, there have been a limited number of translational applications. One study showed that Intrauterine infusion of autologous Tregs may improve live birth rates and reduce early miscarriage rates in patients with RPL, highlighting further investigation in larger clinical trials (80).

Furthermore, understanding the mechanism of fetal-maternal immune tolerance could benefit other areas of immunology where active tolerance to foreign entities is necessary, such as cancer and transplantation. Remarkably, there are striking parallels between pregnancy and cancer, as both trophoblasts and cancer cells invade host tissue, promote angiogenesis, and establish a tolerogenic microenvironment (**Fig. 3**). Trophoblasts constitutively express diverse matrix metalloproteinases (MMPs) and collagenases to facilitate invasion and anchoring within the decidua (102), mirroring the ability of cancer cells to invade and metastasize by exploiting similar invasive mechanisms (103). Moreover, trophoblasts and cancer cells actively induce angiogenesis by expressing VEGF and other angiogenic factors (104,105). A key aspect facilitating immune evasion is antigenic escape, whereby both trophoblasts and cancer cells downregulate classical MHC class I and Ag presentation machinery to circumvent host immune surveillance (106). Tregs and immune checkpoint molecules also contribute to immune tolerance and influence clinical

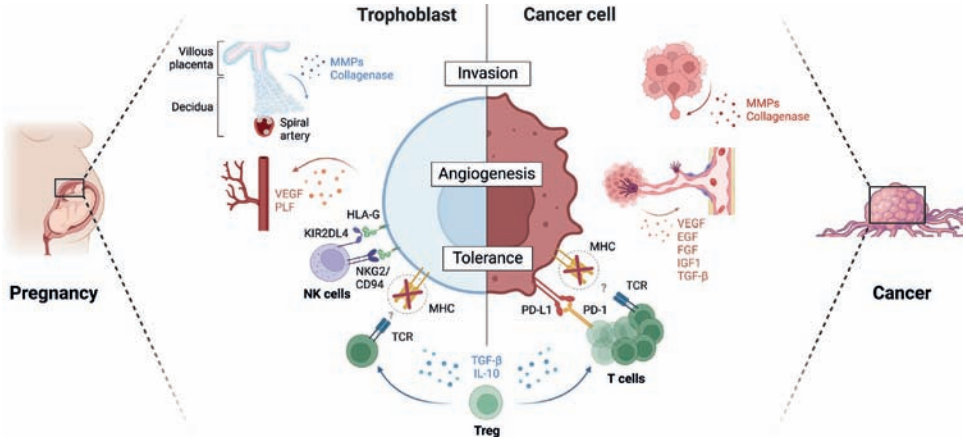

Figure 3. Common molecular features shared between pregnancy and cancer. Both trophoblasts and cancer cells demonstrate invasiveness through the expression of MMPs and collagenases. Additionally, they secrete pro-angiogenic factors including VEGF to induce angiogenesis for proper growth of fetus and cancer. Finally, they evade host immunity by suppressing MHC expression and induce immune tolerance to safeguard their survival and proliferation within the host environment.

outcomes of pregnancy and cancer (79,80,84,85,107). Interestingly, the evolutionary adaptation of trophoblast invasiveness in mammals corresponds with an increased risk of cancer metastasis and gestational disorders (108,109). Given these similarities, elucidating the molecular parallels between pregnancy and cancer could reciprocally inform the development of therapeutic strategies in both contexts. The great success of immune checkpoint inhibitors in cancer therapy exemplifies how future translational impact of insights from pregnancy could advance immunotherapy development. Now, mAb targeting the inhibitory receptors on NK cells including lirilumab (anti-KIR mAb) and monalizumab (anti-NKG2A mAb) are currently under clinical investigation to test their efficacy in cancer immunotherapy (110). In addition, efforts have been made to target tryptophan metabolism for cancer therapy, as the tryptophan-catabolizing enzyme IDO1 is a key tolerogenic factor at the maternal-fetal interface as well as in the tumor microenvironment. Combinations of conventional cancer therapy and various IDO1 inhibitors have shown clear signs of efficacy in clinical trials (111). Finally, while MHC suppression mechanisms of trophoblast remain elusive, given the ongoing investigation into targeting MHC class I inhibitory pathways in cancer (106), these mechanisms could potentially serve as future clinical targets for enhancing anti-tumor immunity.

CONCLUSION

In conclusion, pregnancy represents an immunological paradox where the semi-allogeneic fetus is protected from maternal immune rejection through the active development of robust immune tolerance mechanisms. These multi-layered tolerance mechanisms include physical barriers like the placenta, restricted chemokine expression limiting leukocyte trafficking, lack of sufficient alloantigen presentation, the presence of immunosuppressive cells like Tregs and tolerogenic NK cells, expression of immune checkpoint inhibitors, specific glycosylation patterns conferring immune evasion, and unique metabolic/hormonal adaptations (**Fig. 1**). These cellular and molecular mechanisms are highly interconnected to each component, with one mechanism influencing and reinforcing others through complex regulatory networks in the uteroplacental milieu. Remarkably, many of these

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tolerance pathways mirror strategies employed by cancer cells to promote angiogenesis, tissue invasion, and immune evasion. Clinical studies from patients with gestational complications or cancer also retrospectively reveal the importance of immune tolerance in influencing pathophysiology, diagnosis, treatment, and clinical outcomes. Unfortunately, despite the rising clinical demand, translational applications of insights from maternal-fetal immune tolerance mechanisms have been extremely limited thus far. Therefore, further elucidating the novel mechanistic underpinnings of fetal-maternal immune tolerance holds significant translational potential not only for improving our understanding of gestational complications arising from dysregulated tolerance processes but also for developing novel cancer immunotherapies.

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