



## CORRESPONDENCE

# The mechanisms by which trophoblast-derived molecules induce maternal–fetal immune tolerance

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A successful pregnancy requires the maternal immune system to recognize and tolerate the allogeneic fetus while maintaining defense against infection both systemically and in placental tissues. At the maternal–fetal interface, the trophoblasts in the placenta play an essential role in the suppression of maternal immune rejection of the fetus. To ensure maternal–fetal tolerance and successful placentation, delicate crosstalk is established among fetus-derived trophoblasts, maternal immune cells, and decidual stromal cells (DSCs) during normal pregnancy. Decidual immune cells not only participate in the maintenance of immune tolerance but also regulate the function of trophoblasts to promote fetal growth. An imbalance in this crosstalk may lead to adverse pregnancy outcomes, such as recurrent spontaneous abortion, preeclampsia, preterm birth, intrauterine growth restriction, and infection. Here we outline some of the important discoveries in recent years related to the mechanisms by which trophoblast-derived molecules induce maternal–fetal immune tolerance.

## IMMUNE CELLS AT THE MATERNAL–FETAL INTERFACE

The maternal–fetal interface consists of different immune cells, such as natural killer (NK) cells, macrophages (MΦs), dendritic cells (DCs), T cells, myeloid-derived suppressor cells (MDSCs), B cells, and NKT cells. Trophoblasts have a central role in the control of maternal immune homeostasis mediated by modulating uterine NK cell activity, decidual MΦ polarization into an alternative phenotype, tolerogenic DC differentiation, and regulatory T cell (Treg) induction. Although some research results have been produced, the mechanisms by which trophoblasts exert these functions have not been fully elucidated.

### NK cells

Most leukocytes (up to 90%) that present at the maternal–fetal interface during the first trimester of gestation are decidual NK (dNK) cells. It has become increasingly clear from both human studies and mouse studies that dNK cells differ significantly from peripheral NK (pNK) cells in both phenotype and function. In a healthy state, freshly isolated dNK cells are poorly cytotoxic and skewed toward producing high levels of cytokines and angiogenic factors. Furthermore, dNK cells have been demonstrated to facilitate extravillous trophoblast invasion into the maternal decidua during the first trimester. dNK cells can influence DC function during pregnancy by preventing the maturation of immature DCs, thus protecting trophoblasts from mature DC-

initiated cytotoxic T cell responses, which may play a crucial role in modulating the balance between immune responsiveness and immune tolerance.

### Macrophages

Decidual MΦs (dMΦs) represent approximately 15–20% of decidual leukocytes. MΦs isolated from the decidua during the first trimester mostly exhibit the immunomodulatory phenotype (M2) and contribute to the creation of a tolerogenic immune environment by producing immunosuppressive cytokines to suppress responses to allogeneic fetal antigens, phagocytizing apoptotic trophoblasts to prevent the release of proinflammatory substances, and inhibiting the cytotoxic function of dNK cells. dMΦs also secrete high levels of factors that facilitate placental growth and trophoblast invasion.

### Dendritic cells

DCs comprise approximately 1–2% of the immune cells in the decidua and are scattered through both the decidua basalis (in which trophoblasts are infiltrated) and the decidua parietalis. DCs within the decidual microenvironment exhibit a tolerogenic profile. Human extravillous trophoblasts induce regulatory DCs that poorly induce the proliferation and activation of allogeneic T cells but promote Treg expansion.

### T cells

Decidual T cells comprise 5–15% of placental leukocytes during the first trimester of gestation. Trophoblasts secrete cytokines that regulate the function and differentiation of decidual immune cells and induce epigenetic changes in DSCs, modulating the capacity of DSCs to produce the chemokines necessary for T cell recruitment. Immune tolerance induction by trophoblasts is accomplished not only by directly inhibiting effector T cells and shifting the T helper type 1 (Th1)-type immune response into a Th2-type immune response but also by promoting Treg induction and recruitment. High percentages of Tregs can be found in the decidual tissue, where they suppress fetus-specific and non-specific lymphocyte responses.

### Myeloid-derived suppressor cells

MDSCs, which are capable of inhibiting T cell responses, inducing dNK cell and Treg proliferation and differentiation, increasing the suppressive activity of dNK cells and Tregs, and polarizing CD4<sup>+</sup> T cells toward a Th2 cytokine response, are highly increased in number in the early stage of pregnancy.

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## MECHANISMS BY WHICH TROPHOBLAST-DERIVED MOLECULES INDUCE MATERNAL-FETAL IMMUNE TOLERANCE

During pregnancy, trophoblasts express and secrete a battery of immune inhibitory molecules targeting MΦs, NK cells, DCs, T cells, or MDSCs to sustain maternal-fetal immune tolerance.

### Roles of costimulatory molecules

*T cell immunoglobulin mucin 3 (Tim-3)/galectin-9 (Gal-9).* Tim-3 is an important negative regulatory molecule that induces immune tolerance by interacting with its ligand Gal-9 to modulate the functions of various immune cells. Tim-3, whose expression on decidual CD4<sup>+</sup> T cells is upregulated during pregnancy, promotes a Th2 bias at the maternal-fetal interface. Decreased Tim-3 expression on decidual CD4<sup>+</sup> T cells may be associated with miscarriage. Trophoblasts induce a relatively high frequency of Tim-3<sup>+</sup>CTLA-4<sup>+</sup>CD8<sup>+</sup> T cells during pregnancy; this subset has an active status and produces a relatively high amount of anti-inflammatory cytokines, and a decreased number of these cells and altered function correlate with miscarriage.<sup>1</sup> Tim-3<sup>+</sup> dNK cells display higher interleukin (IL)-4 production and lower tumor necrosis factor-α and perforin production than Tim-3<sup>-</sup> dNK cells. Human trophoblasts induce the transformation of pNK cells into cells with a dNK-like phenotype via the Gal-9/Tim-3 interaction. A decreased percentage of Tim-3<sup>+</sup> dNK cells has been observed in human miscarriage and abortion-prone mouse models.<sup>2</sup> Therefore, Tim-3/Gal-9 signaling pathways may play positive roles in the establishment and/or maintenance of maternal-fetal tolerance.

*Programmed death-ligand 1 (PD-L1)/programmed death-1 (PD-1).* The immune checkpoint molecule PD-L1, a suppressor of T cell activation, is expressed in the syncytiotrophoblasts and cytotrophoblasts in the human placenta and is assumed to induce maternal immune tolerance to fetal antigens via PD-1 receptors on T cells. During human pregnancy, the levels of soluble forms of PD-L1 are reported to be elevated in the serum, and PD-L1 blockade results in spontaneous fetal resorption in pregnant mice.<sup>3</sup> Decidual Tim-3<sup>+</sup>PD-1<sup>+</sup>CD8<sup>+</sup> T cells recognize PD-L1 expressed on extravillous trophoblasts, which provides an inhibitory signal resulting in trophoblast antigen-specific tolerance.<sup>4</sup>

### Roles of cytokines and chemokines

*Interleukin-34.* IL-34 has been shown to bind to the same receptor as macrophage-colony stimulating factor, which has important immunomodulatory roles at the fetal-maternal interface, for example, polarizing dMΦs into an M2-like regulatory phenotype. IL-34 is produced by placental cytotrophoblasts, syncytiotrophoblasts, and DSCs. It is able to polarize blood monocytes into MΦs with an immunoregulatory phenotype (CD14<sup>high</sup>CD163<sup>+</sup>CD209<sup>+</sup>) and a cytokine secretion pattern similar to that of dMΦs observed in vitro, and these macrophages are involved in the establishment of the tolerant milieu found at the fetal-maternal interface.<sup>5</sup>

*Interleukin-35.* IL-35 is expressed in both human first-trimester primary trophoblasts and a trophoblast cell line. Trophoblasts inhibit the proliferation of human naive conventional T cells and convert these cells into IL-35-inducible regulatory T (iT<sub>R</sub>35) cells in an IL-35-dependent manner. Mice with spontaneous abortion have relatively low levels of IL-35 and iT<sub>R</sub>35 cells at the maternal-fetal interface, and exogenous IL-35 induces iT<sub>R</sub>35 cells and prevents immune-induced abortion.<sup>6</sup>

*Receptor activator for nuclear factor-κB ligand (RANKL)/RANK.* Compared with patients with a normal pregnancy, miscarriage patients have abnormally low levels of RANKL/RANK in the villi and decidua. RANKL, which is secreted by human embryonic trophoblasts and maternal DSCs, polarizes dMΦs toward the M2

phenotype and then induces a Th2 bias that promotes maternal-fetal tolerance. Impaired expression of RANKL leads to dysfunction in dMΦs in vivo and increased rates of fetal loss in mice.<sup>7</sup>

*C-X-C chemokine motif ligand 16 (CXCL16)/C-X-C chemokine motif receptor 6 (CXCR6).* A highly coordinated chemokine network not only underlies recruitment of different leukocyte subsets but also orchestrates unique mechanisms of immunomodulation to maintain tolerance. Maternal MΦs can interact with extravillous trophoblasts through the expression of the chemokine receptor CXCR6 and chemokine CXCL16.<sup>8</sup> CXCL16 derived from first-trimester trophoblasts induces the polarization of human M2 MΦs. M2 MΦs polarized by CXCL16 exhibit decreased IL-15 production, which facilitates the inactivation of NK cells. The cytotoxicity of NK cells is attenuated by CXCL16-polarized M2 MΦs, which thereby contribute to the homeostatic, immunotolerant milieu required for successful fetal development.<sup>9</sup>

### Roles of human leukocyte antigen (HLA) molecules

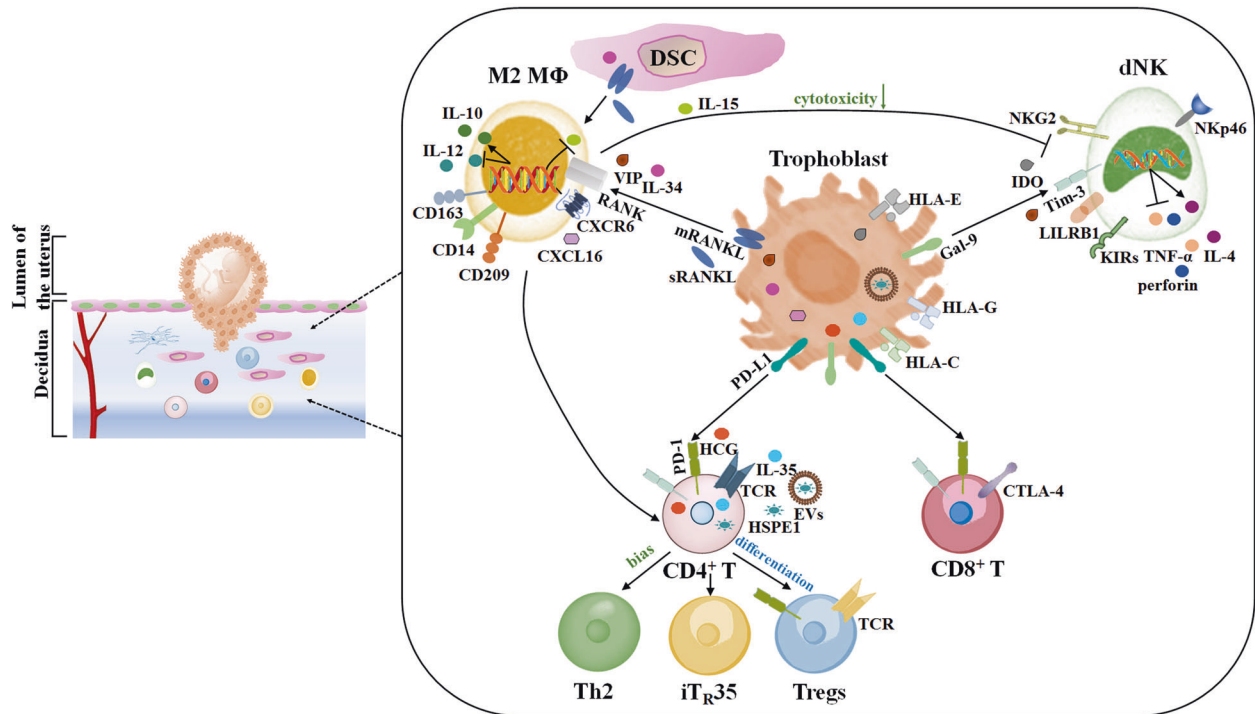
Major histocompatibility complex (MHC) antigen presentation is an important mechanism to prevent immune rejection of the fetus.

*Human leukocyte antigen-C.* HLA-C is a key molecule that can elicit an allogeneic immune response by maternal T and NK cells and for which maternal-fetal immune tolerance needs to be established. HLA-C is also the only classical MHC molecule expressed by extravillous trophoblasts that can present a wide variety of peptides to maternal memory T cells and establish protective immunity. The expression of paternal HLA-C by extravillous trophoblasts provides a target for maternal NK and T cells, but HLA-C expression may also influence how this response is shaped. These dual functions of HLA-C require tight transcriptional regulation of HLA-C expression to balance the induction of tolerance with the induction of immunity. Extravillous trophoblasts can increase PD1<sup>hi</sup> Treg induction via HLA-C and CD3.<sup>10</sup> dNK cells express high levels of killer immunoglobulin receptors (KIRs) specific for HLA-C allelic variants on trophoblasts,<sup>8</sup> and binding between KIRs and HLA-C molecules protects trophoblasts from NK cell cytotoxicity.

*Human leukocyte antigen-G.* A growing body of evidence suggests that HLA-G modulates not only the activity of NK cells but also that of MΦs, T cells, and B cells. Extravillous trophoblasts present HLA-C and the nonclassical HLA-Ib molecules HLA-E and HLA-G on the cell surface. dNK cells express high levels of leukocyte immunoglobulin-like receptor subfamily B member 1, which has a high affinity for HLA-G, and various NKG2 receptors, which recognize HLA-E on extravillous trophoblasts.<sup>8</sup> NK cell inhibition by extravillous trophoblasts can be achieved by HLA-G directly or by HLA-E presenting an HLA-G peptide. HLA-G-induced immune tolerance can occur via the peculiar cellular process trogocytosis, which confers a transient immunosuppressive phenotype upon recipient cells, such as dNK cells and CD4<sup>+</sup> T cells, to allow these cells to play a role in balancing immune tolerance and antiviral immunity at the maternal-fetal interface.<sup>11</sup> Notably, cytokine activation of dNK cells results in the disappearance of internalized HLA-G in parallel with the restoration of cytotoxicity.<sup>11</sup> Coculture of HLA-G<sup>+</sup> extravillous trophoblasts or dMΦs with blood CD4<sup>+</sup> T cells directly increases the proportion of CD25<sup>hi</sup>FOXP3<sup>+</sup> Tregs.<sup>10</sup>

Roles of human chorionic gonadotropin (HCG), indoleamine-2,3-dioxygenase (IDO), vasoactive intestinal peptide (VIP), and heat shock protein family E member 1 (HSPE1)

*Human chorionic gonadotropin.* Trophoblast-derived HCG induces CD4<sup>+</sup>FOXP3<sup>+</sup> Treg differentiation from CD4<sup>+</sup>FOXP3<sup>-</sup>



**Fig. 1** Schematic diagram of trophoblast-derived molecules inducing immune tolerance at the maternal–fetal interface

T cells and increases the suppressive activity of CD4<sup>+</sup>FOXP3<sup>+</sup> T cells through an antigen-independent pathway.<sup>12</sup>

**Indoleamine-2,3-dioxygenase.** Trophoblast-derived IDO can downregulate NKp46 and NKG2D expression and reduce the cytotoxicity of pNK cells; it may also contribute to maintaining dNK cell cytotoxicity at a low level, suggesting an important role for IDO in the maintenance of normal pregnancy.<sup>13</sup>

**Vasoactive intestinal peptide.** VIP secreted by trophoblasts has potent immunomodulatory and trophic effects on adult and embryonic tissues. It has the ability to induce a regulatory/suppressive activation profile in dMΦs and dNK cells. VIP increases IL-10 synthesis and reduces IL-12 activity in dMΦs.<sup>14</sup>

**Hsp10/HSPE1.** Extracellular vesicles exhibit either immunostimulatory properties or immunosuppressive properties depending on their origin and composition. Trophoblast-derived Hsp10/HSPE1 has an important role in Treg differentiation from CD4<sup>+</sup> T cells in vitro, and HSPE1 is a useful marker for Treg subtype characterization.<sup>15</sup>

## CONCLUSION

Maternal immune adaptation is required for a successful pregnancy that avoids rejection induced by embryonic antigens. We need to perform in-depth research to profile placental and uterine cells using a novel high-throughput analysis approach, which will reveal new candidates for trophoblast-mediated immunoregulatory mechanisms. Exploring the functions of trophoblasts will help provide a more complete picture of immune tolerance induction at the maternal–fetal interface, which will have a profound impact on the treatment of immune-related reproductive diseases. The mechanisms by which trophoblast-derived molecules induce immune tolerance are highlighted in Fig. 1.

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## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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