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Indira Guleria and Mohamed H. Sayegh

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BRIEF REVIEWS

Maternal Acceptance of the Fetus: True Human Tolerance

Indira Guleria[1] *and Mohamed H. Sayegh*

Induction and maintenance of immunologic tolerance in humans remains a desirable but elusive goal. Therefore, understanding the physiologic mechanisms of regulation of immune responses is highly clinically relevant for immune-mediated diseases (e.g., autoimmunity and asthma/allergy) and for cell and organ transplantation. Acceptance of the fetus, which expresses paternally inherited alloantigens, by the mother during pregnancy is a unique example of how the immune system reshapes a destructive alloimmune response to a state of tolerance. Understanding the complex mechanisms of fetomaternal tolerance has important implications for developing novel strategies to induce immunologic tolerance in humans in general and for prevention of spontaneous abortion in at-risk populations in particular. *The Journal of Immunology,* **2007, 178: 3345–3351.**

The fetus represents a foreign entity to the maternal immune system, yet this "natural" allograft is not normally rejected. Fifty years ago, Medawar (1) proposed that immunological tolerance should be present during pregnancy to protect against an aggressive maternal alloimmune response directed at the paternal Ags expressed by the fetus. Recurrent pregnancy loss affects 1–3% of all couples, and about half of these cases have no identifiable cause (2). Furthermore, a number of studies associate some pregnancy complications with abnormal maternal immune responses (3, 4). Our understanding of tolerance mechanisms enabling a state of pregnancy is at this stage far from complete. In this review, we will highlight the known mechanisms of fetomaternal tolerance with particular emphasis on recent developments and future directions in the field.

Anatomy of placenta

The tissue most involved in immunoregulation at the maternal-fetal interface is the placenta (Fig. 1). It is comprised of cells of maternal as well as fetal origin, both of which express molecules (HLA-G by trophoblast and Fas ligand (FasL)[2] by maternal decidual cells) that play a role in fetomaternal tolerance. Mouse and human placentas are both composed of layers of cells with distinct functions (5). The outer layer of the mouse placenta, which mediates implantation and invasion into the uterus, is composed of trophoblast giant cells. The layer with analogous function in humans is composed of invasive extravillous cytotrophoblast cells (6, 7). The function of the middle layer of the mouse placenta, the spongiotrophoblast, is largely unknown. However, some of the spongiotrophoblast cells can differentiate into trophoblast giant cells resembling the cytotrophoblast cell columns that anchor the villi of the human placenta (5). The labyrinth layer of the mouse placenta is comparable in function to the chorionic villi of the human placenta. In both the mouse and human placentas, the labyrinthine and villi, respectively, are covered by syncytiotrophoblasts that lie in direct contact with maternal blood.

Mechanisms of fetomaternal tolerance

Many mechanisms protect the fetus from the maternal immune system. These include the expression of nonclassical MHC molecules by trophoblast cells (8, 9), tryptophan catabolism by the enzyme IDO (10), T cell apoptosis (11), and the complement system (12, 13). Recent studies have also documented a role for regulatory T cells (Tregs) (14–17). Finally, we have demonstrated a role for the inhibitory costimulatory molecule, programmed death ligand (PDL)1, in maintenance of fetomaternal tolerance (18). Fig. 1 depicts all of the relevant players (molecules and cell types) that have been shown to play a role in fetomaternal tolerance and their location in the placenta.

Role of MHC

Lack of MHC molecules on trophoblast. Most of the polymorphic MHC class Ia Ags are lacking on the surface of fetally derived trophoblast cells in both mice and humans with some exceptions as cited below (19). In the mouse, some polymorphic fetal MHC class I Ags have been reported on interstitial trophoblasts, mostly in the maternal decidua basalis, but not on endovascular trophoblasts (20). MHC class II Ags are also absent on trophoblast cells (21). The absence of MHC expression

Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Harvard Medical School, Boston, MA 02115

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[1] Address correspondence and reprint requests to Dr. Indira Guleria, Transplantation Research Center, 300 Longwood Avenue, Boston, MA 02115. E-mail address: indira.guleria@tch.harvard.edu

[2] Abbreviations used in this paper: FasL, Fas ligand; DC, dendritic cell; ILT, Ig-like transcript; KIR, killer Ig-like receptor; PDL, programmed death ligand; Treg, regulatory T cell; uNK, uterine NK.

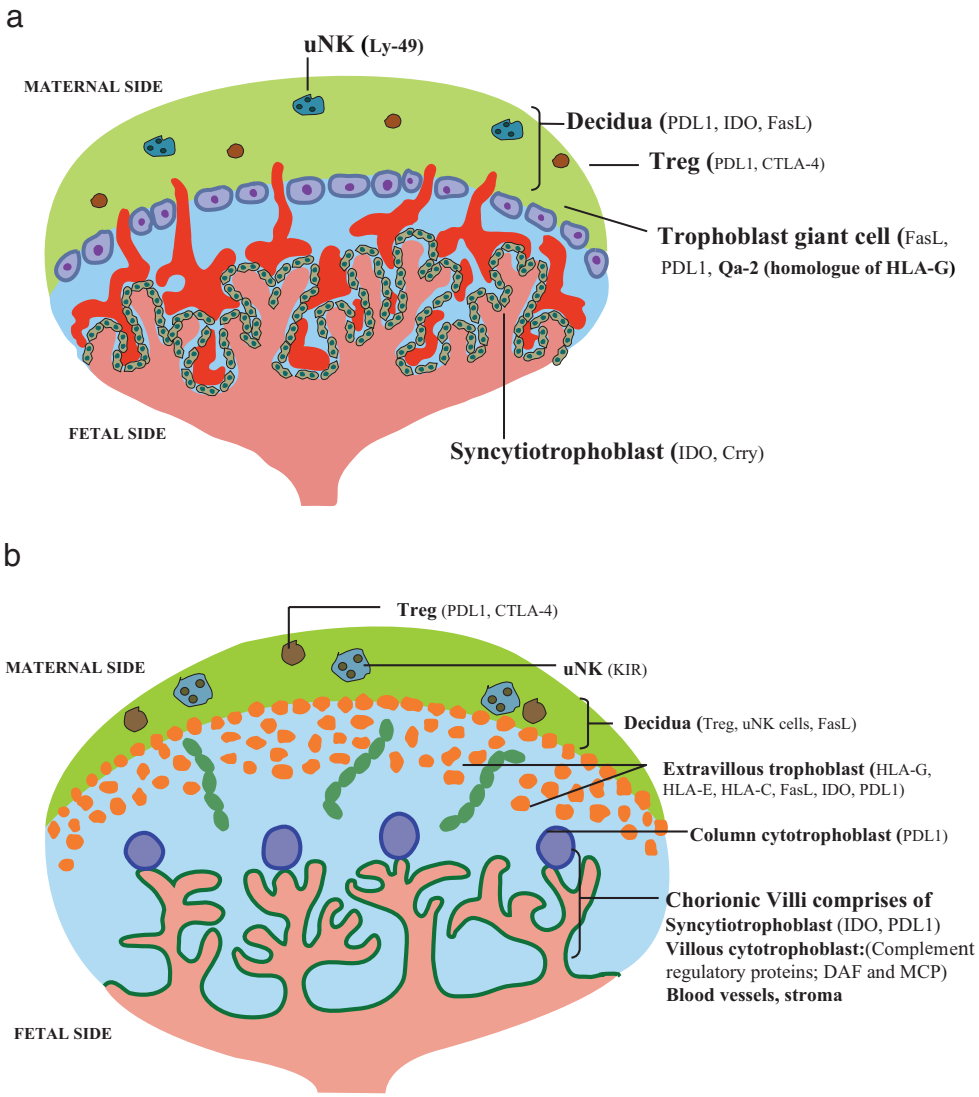

FIGURE 1. Cells and molecules involved in fetomaternal tolerance in mouse and human placenta. Diagrammatic presentation of anatomy of mouse (*a*) and human (*b*) placenta with location of various molecules involved in fetomaternal tolerance (see text for description).

at the maternal-fetal interface is thought to be critical in preventing deleterious maternal immune responses against the fetus. However, transgenic expression of paternal class I MHC molecules did not affect pregnancy rates in multiple studies (22–27), indicating that lack of MHC is not critical in maintaining fetomaternal tolerance.

Trophoblast HLAs and uterine NK (uNK) cells. During pregnancy, there is a general down-regulation of most of the MHC class Ia and class II molecules just before implantation occurs. However, certain class Ib molecules (discussed later) and paternal minor histocompatibility Ags are expressed. Typically, absence of MHC should lead to the escape of trophoblasts from recognition by CTLs while making them susceptible to NK cells (28). However, such cytolysis does not take place, and NK cells found in the placenta are of a distinct type and are called uNK cells. These cells are present in the decidua during the first and second trimester, and they modify the uterine arteries to increase blood supply to the fetoplacental unit (29, 30). uNK cells owing to their increased presence in the decidua and their direct contact with trophoblast have been thought to play a critical role in acceptance/rejection of the fetus (31). The discovery of NK receptors (NKRs) and their ligands on target cells have allowed an understanding of the mechanisms by which NK cells discriminate between target and nontarget cells. These receptors can be activating or inhibitory and belong to largely three families, the killer Ig-like receptor (KIR), the C-type lectin family (CD94/NKGs), and the Ig-like transcripts (ILTs or the leukocyte immunoglobulin-like receptors) (32). *HLA-C* alleles by far seem to be the dominant ligands for KIR2D NKRs. CD94/NKG receptors bind only HLA-E, and KIR3DL1 NKRs bind some HLA-B molecules. Most of MHC class I molecules can bind to ILTs, albeit with different affinities (32). Individual NK cells coexpress multiple combinations of activating and inhibitory receptors, and the repertoire of NKRs vary between different individuals. Studies of NKR polymorphism and the *HLA-C* alleles expressed on the trophoblasts aimed at uncovering the consequences that the various combinations might have on pregnancy outcome have been reported. In one preliminary study, 26 childless couples with two or greater incidences of abortions presenting alloimmune abnormalities were compared with 26 normal couples (33). It was found that the percentage of women with alloimmune recurrent spontaneous abortions lacking inhibitory NKRs carried by their husbands and/or having a limited inhibitory KIR repertoire was much higher than that for women with successful pregnancy outcomes, thereby arguing for a role of the

inhibitory KIRs in protecting the fetus. However, Hiby et al. (34) on their study of the role of the KIR-HLA-C system in the prevalence of pre-eclampsia made a quite different observation. They found that mothers that were of the haplotype that lacked activating receptor, i.e., contained only the inhibitory receptors for a particular (*C2*) allele of *HLA-C*, were found to be at greatly increased risk of pre-eclampsia when carrying fetus with the *C2* allele. They concluded that in the absence of an activating KIR, signaling through the inhibitory KIR leads to impaired uNK cell-mediated modification of uterine arteries, resulting in decreased blood supply and defective placentation. In this case, signaling through inhibitory receptors in NK cells appeared to be detrimental to the survival of the fetus. Therefore, one can assume that a complete understanding of the full range of interactions between the HLA molecules on the trophoblasts and the numerous NKRs on uNK cells, and their implications for fetomaternal tolerance will require a great deal more study.

Trophoblast class Ib HLAs. Extravillous cytotrophoblast tissue expresses nonclassical HLA Ib genes, *HLA-E*, *HLA-F*, and *HLA-G* (9, 19). A number of immunomodulatory functions have been ascribed to HLA-G (35). HLA-G inhibits both CTL responses and NK cell functions (36–38). APCs transfected with HLA-G can prevent the proliferation of $CD4^+$ T cells (39). In addition, $CD8^+$ T cell apoptosis can be induced by soluble HLA-G through the Fas/FasL pathway (37, 40). Recently, it has been shown that HLA-G expression may promote survival of cardiac allografts and that a splice variant of HLA-G promotes allograft acceptance through induction of Tregs (41, 42). The interaction of HLA-G with KIR-related leukocyte Ig-like receptors (also called ILT, leukocyte immunoglobulin-like receptor, and CD85) expressed on dendritic cells (DCs) can also have an indirect influence on immune responses (43, 44). This interaction can render the DCs tolerogenic as they fail to stimulate T cells leading to anergy, have reduced expression of the costimulatory molecules CD80 and CD86, and can facilitate the generation of $CD4^+CD25^+$ Tregs (45).

The role of HLA-G in fetomaternal tolerance remains unclear. In nonhuman primates, such as baboons, placentas generate transcripts from a gene named *Paan-AG*, which has structural similarities to *HLA-G* and is alternatively spliced in a similar manner to *HLA-G* (35, 46, 47). Thus, studies in baboons may turn out to be important in defining a clear role for HLA-G in fetomaternal tolerance. A clear homology of HLA-G is absent in mice, although the molecule Qa-2 is somewhat similar to it (48).

HLA-G in humans is thought to facilitate expression of HLA-E (homologous to murine Qa-1b). Inhibition of NK cell activity takes place upon interaction of HLA-G and HLA-E with the CD94/NKG2 receptor on NK cells (49). The initial belief in the HLA-G field was that it is HLA-G that binds directly to NK cells and inhibits NK cell killing. New data suggest that HLA-G facilitates this process of inhibition of NK cell killing by recruiting HLA-E to the cell surface (8). A leader peptide of HLA-G forms a complex with HLA-E on the trophoblast cell surface and binds to CD94-NKG2. This trimeric complex then binds to NK cells and leads to inhibition of NK cell activity (29). The function of HLA-F is not known at present.

Alloantigen shedding. During the third trimester of normal pregnancy, several grams of dying placental trophoblast is shed daily into the maternal circulation (50–52). However, an increased rate of placental syncytiotrophoblast shedding over normal has been proposed to be related to pre-eclampsia (50, 52, 53). An important role for allogeneic trophoblast shedding and the presentation of these shed paternal Ags by maternal APCs during normal pregnancy has been proposed to assist in establishing maternal immune tolerance to the fetal allograft (25). How alloantigen shedding interacts with other mechanisms of fetomaternal tolerance, including apoptosis and regulation (see below), requires further investigation.

T cell apoptosis

It has been postulated that the establishment of immune privilege at the implantation site is a result of, at least in part, clonal deletion of immune cells that recognize paternal Ags present in the placenta (11, 22). This is believed to be mediated by the expression of FasL on fetal trophoblast or maternal decidual cells (54, 55). In support of a role for maternal FasL, it has been demonstrated that maternal immune tolerance to the *gld* fetus (lacking functional FasL) is maintained (24). Fetus-derived FasL has also been shown to be essential for deletion of allospecific maternal T cells during pregnancy (56). However, the role of Fas-FasL in fetomaternal tolerance has been challenged by some recent studies, where FasL was shown to promote allograft rejection rather than tolerance (57, 58). Two studies by Abrahams et al. (59) and Frangsmyr et al. (60) have independently reported that FasL formed in the microvesicles of trophoblast can compete with the classical surface FasL on these cells, thereby promoting fetal rejection. Therefore, a better understanding of the role of fetal and/or maternal FasL expression and the degree it contributes to fetomaternal tolerance will require further studies.

Immunoregulation

Tregs. Naturally arising $CD4^+$ T cells constitutively expressing the IL-2R α-chain (CD25) are recognized to be involved in the regulation of immune responses and play indispensable roles in the maintenance of natural self-tolerance (61, 62). In the mouse, it has recently been reported that $CD4^+CD25^+$ Tregs expand during pregnancy and are required to prevent immunological rejection of the fetus (14). In another study, Zenclussen et al. (15) demonstrated rescue of allogeneic pregnancy upon transfer of Tregs from normal pregnant mice to abortion-prone mice. The data in humans are consistent with a similar function for Tregs in mouse pregnancy. High-level expression of CD25 is considered necessary for suppressive function of human as compared with mouse Tregs (63). It has been reported that decidual and/or peripheral blood $CD4^+CD25^{+high}$ T cells increased during early pregnancy (16, 64) and then dropped in the postpartum period (17). Taken together, these studies strongly support the notion that pregnancy-induced Tregs play an important role in maintaining tolerance to the fetus. However, the mechanisms of their generation/expansion, specificity, and trafficking (e.g., the roles of alloantigen shedding, hormones, and chemokines) and precisely how they function in vivo remain unknown. In the study by Aluvihare et al. (14), protective Tregs could be generated in pregnant mothers mated with syngeneic males, suggesting that paternal alloantigen may not be necessary to generate Tregs. Estrogen treatment and pregnancy both induced FoxP3 protein expression to a similar degree both in vitro and in vivo, suggesting that high estrogen levels during pregnancy may help maintain fetal tolerance by

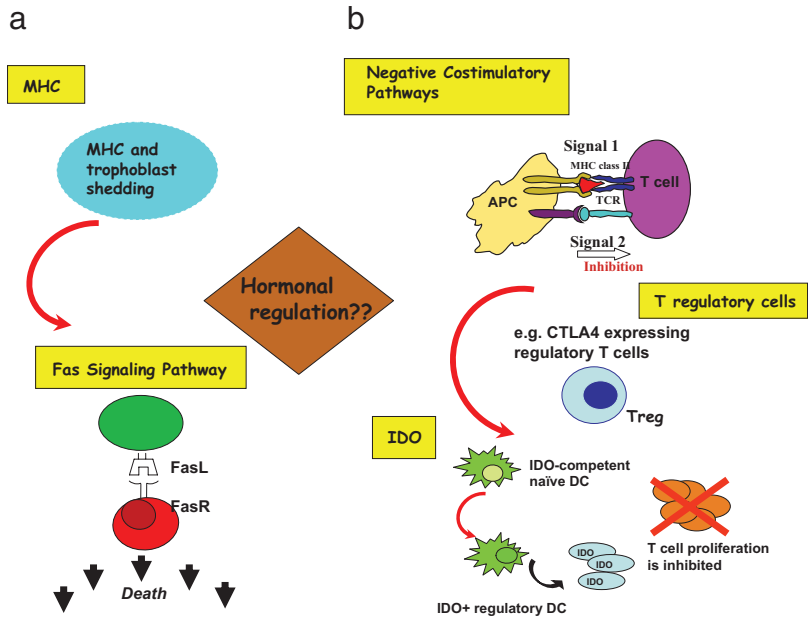

FIGURE 2. Interactions between multiple mechanisms of fetomaternal tolerance. *a*, MHC-associated HLA-G could possibly lead to apoptosis of allogeneic T cells via Fas-FasL pathway. *b*, Tregs are critical components in the maintenance of peripheral tolerance to tissue-specific self-Ags. Negative costimulatory molecule CTLA4-expressing Tregs can induce naive DCs to produce IDO from now referred to as IDO$^+$ regulatory DC. IDO can lead to inhibition of T cell proliferation by catabolizing tryptophan, an amino acid essential for T cell survival. Hormonal regulation of Tregs and IDO have been recently reported, but other molecules/pathways needs to be explored.

promoting regulation (65). Trophoblast-derived chemokines have also been implicated (63). It is reported that CD4$^+$CD25$^+$ Tregs express chemokine receptor CCR4, which mediates trafficking of these cells to tumor cells and macrophages that produce its ligand CCL22 (66). Therefore, the presence of CCL22 during pregnancy (67) might induce the accumulation of CD4$^+$CD25^{+high} Tregs in the decidua (63). Recently, it was proposed that Tregs could also modify the function of APCs through CTLA4-B7 (CD80/CDC86) signaling (68). CTLA4 expression on CD4$^+$CD25$^+$ Tregs up-regulates IDO expression on DCs (69), and it was shown that IDO-expressing "immunoregulatory" DCs play important roles in immunosuppression. Many studies have demonstrated an important role for IDO during pregnancy (10, 70), as discussed below, providing a potential link among Tregs, IDO, and fetomaternal tolerance.

CD8$^+$ Tregs, which are not class I MHC restricted (express mucosal markers CD101 and CD103), but require costimulation through a member of the carcinoembryonic Ag family, present on early gestation trophoblasts are a new emerging class of regulatory cells (71), and their role, if any, in fetomaternal tolerance should unfold in the future. CD8$^+$ Tregs expressing CD103 have been recently shown to have immunoregulatory functions in the eye, another immunoprivileged site (72).

Indoleamine 2,3-dioxygenase. IDO is an enzyme that degrades the essential amino acid tryptophan and can generate downstream tryptophan metabolites. It is expressed on trophoblastic giant cells in mice and both extravillous trophoblasts and villous trophoblasts in humans (73), where it could possibly inhibit maternal T cell activation by depriving T cells of tryptophan. Interestingly, serum tryptophan level has been shown to decrease from the first trimester of human pregnancy (74). Pharmacologic inhibition of IDO activity during murine pregnancy was also shown to results in maternal T cell-mediated rejection of allogeneic but not syngeneic concepti (10). Results in IDO-deficient mice, however, showed that genetic deletion of IDO resulted in normal litter size relative to those of their IDO-sufficient counterparts (75). This suggests that alternative mechanisms can take over its protective role in its absence (75). Also to be noted is that mammals have a second enzyme, tryptophan dioxygenase, that promotes tryptophan catabolism and may compensate for the lack of IDO (76, 77).

IDO may also have an indirect effect on immune response by affecting the nature and function of IDO-expressing APCs. IDO might functionally alter DCs, either by decreasing their APC function or by up regulating expression of suppressive ligands (e.g., PDL1 or CD95 ligands) or by triggering the secretion of immunoregulatory cytokines (e.g., IL-10 or TGF-β). Recent studies suggest that certain CTLA4$^+$ Tregs can induce IDO expression (78). Furthermore, expression of HLA-G on DCs can be induced by IDO, thereby suggesting that these two molecules probably interact in establishing fetomaternal tolerance (79).

B7 family of costimulatory molecules

Optimal T cell activation requires the engagement of the TCR with a cognate MHC$^+$ peptide complex and a "positive" T cell costimulatory signal mediated by one of several other cell surface molecules (80, 81). Some T cell costimulatory molecules provide "negative" signals and function to regulate immune responses (82). Typically, these are inducible molecules that are expressed after activation and function to terminate immune responses.

Blockade of positive costimulatory signals such as CD80 (B7-1) and CD86 (B7-2) was shown to inhibit maternal rejection of the allogeneic fetus in abortion-prone CBA/JxDBA/2 matings (83).

The prototypic negative costimulatory molecule CTLA4 is expressed in fetal tissues at the maternal-fetal interface throughout gestation (84). Polymorphism in the *CTLA4* gene has recently been suggested to confer susceptibility to recurrent spontaneous abortion in Chinese women (85).

The programmed death-1 receptor and its ligands, PDL1 and PDL2, define a novel regulatory costimulatory pathway that plays an important role in peripheral tolerance (80, 82, 86). During pregnancy, PDL1 (B7-H1) is present on all trophoblast

populations, and PDL2 (B7-DC) is expressed by the syncytiotrophoblast in early pregnancy (87). In mice, PDL1 is expressed in the decidua basalis, the maternal part of the placenta (18). We have recently shown that PDL1 and PDL2 are expressed at the fetomaternal interface and that PDL1 is necessary for maintaining fetomaternal tolerance (18). Blockade as well as deficiency of PDL1 resulted in decreased fetal survival and a shift to Th1 cytokines in the periphery (spleen) as well as locally in the placenta. Th2 immune responses have been shown to be important for maintenance of healthy pregnancy (88). In addition, our studies have also revealed a possible link between PDL1 and Tregs in a skin transplantation model (89), but the link in fetomaternal tolerance is under investigation. Very recently, it has been shown that both estrogen and pregnancy markedly enhanced programmed death-1 expression in several types of APCs, especially DCs (90). The functional significance of these expression patterns and how they relate to mechanisms of fetomaternal tolerance in humans require further investigation.

Role of complement and complement regulators

Successful pregnancy is maintained by the expression of complement regulatory proteins expressed on trophoblast, which prevent damage inflicted by complement activation. Decay-accelerating factor (CD55) and membrane cofactor protein (CD46) are complement regulatory proteins expressed on human trophoblast and are crucial for sustaining pregnancy (91). Crry is a functional homolog of CD55 and CD46 and is found only in rodents. Deficiency of mouse Crry leads to embryonic death (92). Crry-deficient embryos suffer from increased C3 deposition through the alternate pathway and concomitant inflammation within the developing placenta. Complement activation is also required in a mouse model of recurrent fetal loss associated with anti-phospholipid Abs, a condition characterized by increased rate of spontaneous abortion (93). Interestingly, a relationship may exist between complement and IDO, and complement and PDL1 as mice treated with inhibitors of these pathways had deposition of complement in the placenta. It remains to be established whether complement deposition is the cause of fetal death in these treated mice or if it is just one of the contributing factors.

Conclusions and future directions

Multiple mechanisms have been implicated in mediating fetomaternal tolerance (Fig. 2). The contribution of each mechanism and the potential interactions among the various pathways are just beginning to emerge. Increasing attention is being focused on the role of Tregs that may serve as a common pathway ultimately leading to maintenance of tolerance. The relationship of Tregs function with Treg costimulatory pathways (CTLA4 and PDL1) requires further investigation. Another important question is to what extent these immunoregulatory mechanisms are subject to hormonal regulation in vivo. Answers to these questions, in addition to providing further insights into this most unique example of human tolerance among nature's creation, will also enhance our ability to manipulate cellular pathways for better outcome in disease situations such as in recurrent spontaneous abortion, transplantation, and autoimmunity.

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Disclosures

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