What is the role of regulatory T cells in the success of implantation and early pregnancy?

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Abstract

Problem The immune system is well controlled by the balance between immunostimulation and immunoregulation. CD4⁺CD25⁺ regulatory T (Treg) cells and an enzyme called indoleamine-2, 3-dioxygenase (IDO) mediate maternal tolerance of the allogeneic fetus. Treg cells, therefore, may prevent early pregnancy loss due to maternal ‘rejection.’

Methods The latest understanding of tolerance during pregnancy is reviewed.

Results and conclusions Recent data show that CD4⁺CD25⁺ Treg cells play essential roles in the induction and maintenance of tolerance, and that they augment the IDO activity in dendritic cells and macrophages. Therefore, CD4⁺CD25⁺ Treg cells and IDO enzyme may cooperate in the induction of tolerance during pregnancy. Treg deficiency is associated with very early post-implantation loss and spontaneous abortion in animal models, and low Treg levels are associated with recurrent miscarriages in humans.

Keywords CD4⁺CD25⁺ regulatory T cell · CTLA-4 · Decidua · IDO · Miscarriage · Preeclampsia

Introduction

The fetus is a semi-allograft to the maternal host, and maternal T cells are aware of fetal alloantigens [1]. Assisted reproductive technologies (ART) have led to great advances in reproductive medicine. In donated embryo cases, the fetus can be a complete allograft, with no maternal genes whatsoever, but successful pregnancy is still established and miscarriages do not occur at increased rates [2, 3]. The risk of preeclampsia in these cases, however, increases up to ten times compared to a traditional pregnancy, where the fetus represents only in 50% foreign paternal genes [3]. This clinical finding suggests that a mechanism, which leads to tolerance to (paternal) alloantigens, is present and important. Such a tolerance system ensures normal pregnancy for the semi-allogeneic fetus, but appears inadequate in fully allogeneic pregnancies. Therefore, immunomaladaptation manifests as preeclampsia at a much higher prevalence.

It has recently been reported that T cell responses are regulated by CD4⁺CD25⁺Foxp3⁺ regulatory T (Treg) cells, and that these cells play very important role in immunotolerance [4, 5]. We firstly reported that the proportion of CD4⁺CD25⁺ T cells in the pregnancy decidua was high [6], but at that time the concept of CD4⁺CD25⁺ Treg cells had not been recognized. In maintenance of allogeneic mouse pregnancy, CD4⁺CD25⁺ Treg cells play an essential role in the induction of maternal tolerance to the semi-allogeneic fetus [7, 8]. An enzyme called indoleamine 2, 3-dioxygenase (IDO) may also mediate maternal tolerance to the allogeneic fetus [9]. IDO is expressed in dendritic cells (DCs), macrophages (Mφ), giant trophoblasts in mice, and in extravillous trophoblast (EVT) and villous trophoblast in humans [10–12]. Recent data demonstrated that CTLA-4, which is expressed on CD4⁺CD25⁺ Treg cells, enhances the

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IDO activity in DC and M ϕ [13, 14]. These findings demonstrate that CD4⁺CD25⁺ Treg cells and IDO enzyme may cooperate closely in the induction of adequate tolerance during pregnancy.

In this review article we discuss the role of CD4⁺CD25⁺ Treg cells and IDO expression in DC and M ϕ for successful pregnancy, and we analyse these immune system in selected pregnancy complications to better understand the pathophysiology of miscarriage and preeclampsia.

Paternal antigen-specific tolerance is induced during pregnancy

When H-2K^b-recognizing T cell receptor-transgenic (Des-TCR) female mice were mated with H-2K^b expressing male mice, H-2K^b recognizing CD4⁺ T and CD8⁺ T cells in Des-TCR mice dramatically decreased during pregnancy [1]. Interestingly, CD8⁺ T cells, which react with H-2K^b, completely disappeared from placental and embryonic tissues during H-2K^b allogeneic pregnancy. This systemic clonal deletion of T cells was also observed in thymectomized Des-TCR mice, suggesting that clonal deletion occurred at an extrathymic region. It was further shown that female mice accept paternal MHC antigen-expressing allogeneic tumor graft during pregnancy, and that these tumors were rejected three weeks after delivery [1], suggesting that paternal antigen-specific tolerance was limited to pregnancy. Fas ligand (Fas L) and HLA-G molecules on trophoblasts may also play a role in clonal deletion (Fig. 1, middle box). Fas L promotes apoptosis of activated

lymphocytes, expressing Fas (CD95) on their surface. Jiang et al. reported that the Fas/Fas L system plays in mice during pregnancy an important role in clonal deletion at the materno-fetal interface [15]. Indeed, pregnancy in Fas L-lacking *gld* mice shows extensive infiltration of lymphocytes at the materno-fetal interface [16]. In humans, Fas L is expressed on villous trophoblast and extravillous trophoblast (EVT) [17], suggesting that maternal activating T cells might be deleted by the Fas/Fas L system (Fig. 1, middle box).

HLA-G is only expressed in the placenta and thymus in humans, and soluble HLA-G1 is produced by syncytiotrophoblast, and probably by EVT. Interestingly, soluble HLA-G1 induces apoptosis of activated CD8⁺ T cells and this is mediated by the Fas/Fas L system (Fig. 1) [18]. Soluble HLA-G1 may also play an important role in the clonal deletion of activating cytotoxic CD8⁺ T cells, which react to paternal antigens. Soluble HLA-G1 also regulates the proliferation of CD4⁺ T cells in mixed lymphocyte reactions (Fig. 1, middle box) [19]. Decreased HLA-G expression in trophoblast in cases preeclampsia has been reported [18], and may account for inadequate induction of tolerance in preeclampsia.

Expression of major histocompatibility complex (MHC) on human trophoblasts

Syncytiotrophoblasts has direct contact with maternal circulating lymphocytes in the intervillous space. Interestingly, syncytiotrophoblast does not express MHC class I or

Fig. 1 Induction of maternal MHC antigen-specific tolerance during pregnancy in human. Villous trophoblasts do not express MHC class I antigens or MHC class II antigens, but MHC class II antigens such as HLA-DR, and DQ are expressed on the endoplasmic reticulum (ER) in trophoblastic cell debris. These antigens may be presented by maternal dendritic cells, and activated maternal CD8⁺ T cells may be deleted by sHLA-G₁ or the Fas/Fas ligand system. On the other hand, fetal antigen-recognized maternal CD4⁺ T cells may differentiate to CD4⁺CD25⁺ Treg cells. Furthermore, CEA associated with CD1d on trophoblasts may induce regulatory CD8⁺ T cells, and PD-L1 on trophoblasts may induce tolerance by the PD-L1/PD-1 system

MHC class II antigens in humans (Fig. 1). On the other hand, EVT, which invades maternal decidual tissue, expresses polymorphic HLA-C and monomorphic HLA-G, HLA-E and HLA-F (Fig. 1). The MHC-class II antigen is not expressed on EVT or villous trophoblasts.

Under such circumstances, maternal immunocompetent cells do not make effective contact with paternal allo-antigen-expressing cells and paternal antigen-specific tolerance does not seem to be required (in the maintenance of allogeneic pregnancy). Several researchers, indeed, question whether MHC-class I or MHC class II-specific tolerance is present systemically during human pregnancy [20].

Many recent studies have demonstrated that the placenta is not as impenetrable a physical barrier, preventing the exchange of cells between the mother and fetus, as has been thought. Microchimerism between mother and fetus is common. As a result, fetal cells can be detected in the maternal circulation and maternal cells can also be detected in newborns for long time (Fig. 1) [21].

Furthermore, a massive amount of trophoblastic cell debris (~several grams per day in term pregnancy) is released into the maternal circulation [22]. This cell debris is largely derived from senescent trophoblastic apoptotic cells. Recent data showed that MHC-class II antigens such as HLA-DR and DQ, but not DM, are expressed on the endoplasmic reticulum (ER) in trophoblastic cell debris (Fig. 1, upper box) [23]. When maternal DC takes up MHC-class I- and MHC-class II antigen- expressing fetal mononuclear cells, and MHC-class II- expressing trophoblastic cell debris, these antigens are presented to maternal CD8⁺ T cells and CD4⁺ T cells (Fig. 1). Recent reports have also demonstrated that ingestion of apoptotic cells by phagocytes, such as DC and M ϕ , results in active immunosuppressive and anti-inflammatory responses [16, 24].

MHC-class II antigens may be presented to CD4⁺ T cells, and as a result, these cells might differentiate CD4⁺CD25⁺ Treg cells in the periphery (Fig. 1, middle box). MHC-class I antigens may be recognized by maternal CD8⁺ T cells, and these activating CD8⁺ T cells could be deleted by the Fas/Fas L, or soluble HLA-G1 systems at the materno-fetal interface (Fig. 1, middle box).

A subset of CD8⁺ Treg cells may be activated by the combination of the non-classical class I molecule CD1d and co-stimulatory molecules of the carcinoembryonic antigen (CEA) family (Fig. 1, lower box). Shao et al. reported that human placental trophoblast activates a clonal population of CD8⁺ T cells with a regulatory function in antibody production, but not in the allogeneic MLR response [25]. These CD8⁺ Treg cells display selective usage of the TCR gene V β 9, and this stimulation requires co-stimulation through a member of the CEA antigen family present on early gestation trophoblasts. These findings support the idea

that specific tolerance may present at the materno-fetal interface during pregnancy.

The role of CD4⁺CD25⁺ Treg cells for induction of tolerance during pregnancy

Aluvihare et al. reported that CD4⁺CD25⁺ Treg cells mediate maternal tolerance to the allogeneic fetus in mice [7]. BALB/C nu/nu mice lack a thymus, so, they do not have CD4⁺CD25⁺ Treg cells. When BALB/C-derived immunocompetent lymphocytes are transferred, both conventional T cells and CD4⁺CD25⁺ Treg cells are provided to the nu/nu recipients. When these female mice were mated with C57BL/6 male mice (allogeneic pregnancy), they showed normal pregnancy (Fig. 2a). However, when BALB/C-derived CD25[−] lymphocytes were transferred into BALB/C nu/nu female mice, the females had conventional T cells, but not CD4⁺CD25⁺ Treg cells. These mice showed abortion in allogeneic pregnancy, but normal pregnancy in syngeneic pregnancy matings (Fig. 2a). Interestingly, most of the pregnancy loss of semi-allogeneic embryos occurred very early after implantation, but a few embryos survived long enough to be identified as classical abortions (resorptions).

It was also reported that peripheral blood-, pelvic lymph node-, inguinal lymph node-, and splenic- CD4⁺CD25⁺ T cells increased from an early stage of pregnancy, beginning on day 2.5 of pregnancy [7]. This increase was also observed in syngeneic pregnant mice, suggesting that increased CD4⁺CD25⁺ Treg cells may be alloantigen non-specific. Zenclussen et al. reported that adoptive transfer of CD4⁺CD25⁺ Treg cells from CBA/J $\times$ BALB/c pregnant mice prevented fetal rejection in a CBA/J $\times$ DBA/2 abortion model (Fig. 2b), but only if transferred before day 4.5 of pregnancy [8]. On the other hand, the transfer of CD4⁺CD25⁺ Treg cells from non-pregnant mice failed to prevent abortion (Fig. 2b). These findings suggest that pregnancy-specific, or alloantigen-specific CD4⁺CD25⁺ Treg cells may play an important role in successful semi-allogeneic pregnancy.

Kallikourdis et al. reported that CCR5 expressing CD4⁺CD25⁺ Treg cells are effector regulatory T cells [26]. Interestingly, CCR5⁺ effector- regulatory T cells preferentially accumulate in the pregnant uterus [26]. Also, anti CD25 monoclonal treatment on day 0.5–2.5 of pregnancy induced implantation failure in an abortion model [8] and in a BALB/c $\times$ C57BL/6 semi-allogeneic pregnancy model [27] (Fig. 2b and c), suggesting that CD4⁺CD25⁺ Treg cells play essential roles before or at implantation in semi-allogeneic pregnancy as well as at later times in gestation.

Although these findings strongly support the idea that CD4⁺CD25⁺ Treg cells are essential for successful

Fig. 2 The role of $CD4^+CD25^+$ Treg cells in the induction of tolerance during pregnancy. When $CD25^+$ cells are absent and conventional T cells are present, BALB/C nu/nu mice show abortion in an allogeneic pregnancy model, and show normal pregnancy in a syngeneic pregnancy model (a). In an abortion-prone model, adoptive transfer of $CD4^+CD25^+$ T cells from pregnant CBA/J mice on day 0 prevents abortion, but adoptive transfer of these $CD4^+CD25^+$ T cells on day 4.5 does not prevent abortion (b). Adoptive transfer of $CD4^+CD25^+$ T cells from non-pregnant mice on day 0 does not prevent abortion (b). Administration of an anti CD25 monoclonal antibody on day 0 induces abortion in an allogeneic pregnancy model (c)

pregnancy, there are some problems which must be resolved. An anti-CD25 monoclonal antibody was used to reduce the $CD4^+CD25^+$ Treg cells, but these antibodies may also reduce activated CD25-expressing $CD4^+$ and $CD8^+$ T cells. It will be necessary to confirm the role of $CD4^+CD25^+Foxp3^+$ Treg cells during pregnancy, using bacterial artificial chromosome (BAC)-transgenic mice, known as “depletion of regulatory T cell” (DEREG) mice [28] or diphtheria toxin receptor (DTR) fused $Foxp3$ transgenic mice [29].

In humans, $CD4^+CD25^+$ Treg cells are heterogeneous, and only $CD4^+CD25^{high}$ T cells express immunoregulatory function [30]. In human pregnancy, circulating $CD4^+CD25^{high}$ Treg cells increase in the early pregnancy period, and reach the maximum level in the second trimester, decreasing to a non-pregnant level after delivery [31, 32]. Interestingly, the population of $CD4^+CD25^{high}$ cells in early pregnancy decidua is about three times higher, compared to that in peripheral blood [31, 33]. The population of $CD4^+CD25^{high}$ Treg cells in the decidua and peripheral blood of miscarriage cases decreases to non-pregnancy levels [31], suggesting that $CD4^+CD25^{high}$ Treg cells play a significant role in the maintenance of human pregnancy.

$Foxp3$ is a master gene for differentiation of $CD4^+CD25^+$ Treg cells [34]. Estrogen augments $Foxp3$ expression in vitro and in vivo, and estrogen treatment

increased the number and function of $CD4^+CD25^+$ Treg cells [35, 36]. A high estrogen level during pregnancy may have some role in the expansion of $CD4^+CD25^+$ Treg cells. Jasper et al. reported that the expression of $Foxp3$ mRNA in the mid-secretory phase of endometria with primary unexplained infertility was very low [37]. Reduced endometrial $Foxp3$ mRNA may impair differentiation of uterine $CD4^+CD25^{high}$ Treg cells, resulting in implantation failure. Uterine $CD4^+CD25^{high}$ Treg cells may, therefore, be a key player in successful implantation.

$CD4^+CD25^+$ Treg cells express L-selectin, cutaneous lymphocyte antigen (CLA) and chemokine receptors, such as CCR4 and CCR8 [38–40]. Interestingly, the expression of L-selectin on peripheral blood $CD16^-CD56^{bright}$ NK cells up-regulates in the preovulatory phase (day 10–12), and L-selectin plays a very important role in the homing to the uterus [41]. The same mechanism may be used for the migration of $CD4^+CD25^+$ Treg cells into the uterus. Indeed, there was a 90% reduction in $CD4^+CD25^+$ Treg cells in peripheral lymph nodes in L-selectin $-/-$ mice, with a compensatory increase in $CD4^+CD25^+$ Treg cell numbers in the spleen [38].

CLA expressed on $CD4^+CD25^+$ Treg cells may also play a role in the migration of Treg cells into the uterus, while several chemokines for CCR4 and CCR8 may also accumulate $CD4^+CD25^+$ Treg cells in the uterus.

In a recent study, Treg cells were divided into a highly-suppressive $CCR5^+$ and a low-suppressive $CCR5^-$ subpopulation. $CCR5^+$ Treg cells accumulated and CCL4 (a ligand of $CCR5$) expression was increased in the gravid uterus. In addition, when $CCR5$ -deficient Treg cells from $CCR5^{-/-}$ mice were injected into T cell-deficient mice, fetal loss was increased in allogeneic matings [26].

The cross-talk between $CD4^+CD25^+$ Treg cells and IDO expressing DC and $M\phi$

An activation signal is necessary for induction of the regulatory function of $CD4^+CD25^+$ Treg cells (Fig. 3). In this process, antigen presenting cells (APCs), such as DC and $M\phi$, present antigens to $CD4^+CD25^+$ Treg cells. After activation, $CD4^+CD25^+$ Treg cells express chemokine receptor $CCR5$ [26] and surface CTLA-4 [31]. These activated $CD4^+CD25^+$ Treg cells display immunoregulation by cell-to-cell interaction or production of immunoregulatory cytokines, such as TGF- β and IL-10 (Fig. 3).

In the periphery, $CCR5$ is expressed on 10–20% of $CD4^+CD25^+$ T cells. $CD4^+CD25^+CCR5^+$ T cells show marked reduction of T cell proliferation in mice, even in the absence of APCs [26]. Further, $CD4^+CD25^+CCR5^-$ T cells have no ability for immunoregulation in the absence of APCs.

The former studies detected the immunoregulatory function of $CD4^+CD25^+$ T cells in the presence of irradiated APCs. APCs may play an important role in the induction of functional $CD4^+CD25^+CCR5^+$ Treg cells from

$CD4^+CD25^+CCR5^-$ T cells. In the pregnant murine uterus, $CD4^+CD25^+CCR5^+$ Treg cells accumulated in the gravid uterus in allogeneic, but not in syngeneic matings [26], suggesting that Treg cells, which recognize paternal antigens, recruit into the pregnant uterus or uterine DCs induce $CD4^+CD25^+CCR5^+$ Treg cells from $CD4^+CD25^+CCR5^-$ T cells.

In humans, the population of surface CTLA-4 expressing $CD4^+CD25^{high}$ T cells increases in normal pregnancy decidua, but these cells decrease to non pregnancy level in miscarriage cases [31]. Interestingly, surface CTLA-4 expressing $CD4^+CD25^{high}$ T cells do not increase in peripheral blood of pregnant subjects, suggesting that paternal antigen recognizing $CD4^+CD25^{high}$ Treg cells might accumulate in the pregnant uterus in human, and fetal antigen-recognizing decidual $CD4^+CD25^{high}$ Treg cells, which express surface CTLA-4 on their surface, may prevent fetal rejection. Indeed, anti-CTLA-4 antibody treatment inhibited $CD4^+CD25^+$ Treg function in vivo [42].

As shown in Fig. 3, CTLA-4 on $CD4^+CD25^{high}$ Treg cells may induce the tryptophan catabolizing enzyme IDO. When surface CTLA-4 on $CD4^+CD25^{high}$ Treg cells bind to the B7 complex on antigen presenting cells (APCs), IFN- γ production is induced [13, 14]. Following this, the production of IFN- γ enhances the IDO expression by DC or $M\phi$. The expression of CD86, but not CD80, on peripheral blood- and decidual-DC, and $M\phi$, is up-regulated in normal pregnancy, and down-regulated in cases of miscarriage [43].

IFN- γ production by decidual leukocytes, stimulated with CTLA-4/Fc, is dramatically increased in normally

Fig. 3 The mechanisms for immunoregulation by $CD4^+CD25^+$ Treg cells during pregnancy. As a first step, $CD4^+CD25^+$ Treg cells are activated by T cell receptor (TCR) system and CD28-B7 co-stimulatory system. These activated $CD4^+CD25^+$ Treg cells express CTLA-4 and $CCR5$ on their surface, and have an ability for immunoregulation. $CCR5^+$ Treg cells infiltrate toward sites of inflammation such as the fetomaternal interface. $CD4^+CD25^+$ Treg cells manifest immunoregulation by up-regulation of IDO in APCs, cell-to-cell interaction through membrane TGF- β 1, LAG-3, PD-1 and surface galectin-1 or immunoregulatory cytokines such as TGF- β and IL-10

pregnant subjects, but decreased in miscarriage cases [43]. Interestingly, CTLA-4/Fc dramatically augments IDO expression on peripheral- and decidual-DC, but IFN- γ slightly augments IDO expression on DC [43] of pregnant women. IDO expression on DC, after CTLA-4 treatment, is decreased in miscarriage cases [43]. Terness et al. reported that human DCs, by treating the cells with IFN- γ , expressed IDO, but these DCs did not inhibit T cell response [44]. This finding suggests that CTLA-4, expressed on CD4⁺CD25^{high} Treg cells, but not IFN- γ , might in the pregnant uterus induce immunoregulatory DC.

The up-regulated IDO enzyme, induced by CTLA-4, depletes tryptophan at the materno-fetal interface, thereby preventing T cell, and NK cell activation [9–11]. CD4⁺CD25⁺ T and IDO-expressing DCs are thus very important in the maintenance of semi-allogeneic pregnancy.

Another important molecule for cell-to-cell interaction of CD4⁺CD25⁺ Treg cells is cell surface TGF- β 1, which regulates T cell activation and NK cell function [45] (Fig. 3). The role of surface TGF- β has, however, remained controversial, because CD4⁺CD25⁺ Treg cells in TGF- β 1 knockout mice can mediate a suppressor function [46].

Another molecule, Lag-3, may contribute to the suppressive function of CD4⁺CD25⁺ T cells [47], but its suppressive effect is only modest. Recently Garin et al. performed a transcriptomic and proteomic analysis of activated CD4⁺CD25⁺ T cells, and found galectin-1 was selectively up-regulated in CD4⁺CD25⁺ Treg cells [48] (Fig. 3). Galectin-1 is a 14kDa proto-type member of the galectin family and binds carbohydrate moieties in CD45, CD43, CD2, CD3 and CD7. Blockade of galectin-1 binding reduces the inhibitory effects of human and mouse CD4⁺CD25⁺ Treg cells, and CD4⁺CD25⁺ T cells, obtained from galectin-1 homozygous null mutant mice, showed reduced regulatory activity [48]. Interestingly, uterine CD16⁺CD56^{bright} NK cells over-express galectin-1, and this production is regulated by sex hormones [49]. Surface galectin-1 on CD4⁺CD25⁺ Treg cells might be regulated by sex steroid hormones. Galectin-1, expressed on CD4⁺CD25⁺ Treg cells and uterine NK cells, may closely co-operate in the immunoregulation at the materno-fetal interface.

The CD28-B7 and immune costimulatory molecule-ICOS-B7h pathways promote T cell activation, whereas CTLA-4 and PD-1-PDL pathways lead to down regulation of T cell activity. The PD-1 receptor is a CD28 family inhibitory receptor, and it is involved in the regulation of peripheral tolerance [50]. Interestingly, CD4⁺CD25⁺ Treg cells also express PD-1. The ligand for a PD-1, PDL-1 knockout mouse, results in dramatic abortion of allogeneic, but not of syngeneic pregnant mice [51], suggesting that PD-1-PDL-1 pathways induce maternal tolerance.

In humans, PDL-1 is expressed on syncytiotrophoblasts, cytotrophoblasts and extravillous trophoblasts throughout pregnancy [52]. Trophoblasts may be protected from maternal T cell attack via the PDL-1/PD-1 system, and trophoblasts might regulate CD4⁺CD25⁺ Treg function by the PDL-1/PD-1 system.

To find Foxp3 target genes, DNA microarray and Chip-on-chip technology have been carried out in both mouse and human systems [55–58]. It is possible that other new, and important, molecules in the immunoregulation of CD4⁺CD25⁺ Treg cells will be found by such studies.

Reduced function and reduced number of CD4⁺CD25⁺ Treg cells in complicated pregnancy

We already showed that the number of decidual- and peripheral blood-CD4⁺CD25^{high} Treg cells decrease in cases of miscarriage [31], and Zenclussen et al. reported that the number of CD4⁺CD25⁺ Treg cells decreased in a CBA/J x DBA/2 mouse abortion model [8], suggesting that decreased CD4⁺CD25⁺ Treg cells might cause miscarriages. Recent data demonstrated that recurrent spontaneous abortions cases showed low numbers and deficient function of CD4⁺CD25⁺ T cells [59]. Reduced endometrial CD4⁺CD25⁺ Treg cells may also be a cause for unexplained implantation failure [37].

Recent studies also showed decreased Foxp3 expression in CD4⁺CD25⁺ Treg cells with various immune disorders, such as graft-versus-host disease, myasthenia gravis and multiple sclerosis [60–62]. In a mouse model, in which endogenous Foxp3 gene expression is attenuated in Treg cells (FILIG mice; Foxp3-IRES-luciferase-IRES-eGFP), decreased Foxp3 expression causes immune disease by subverting the suppressive function of Treg cells, and converting Treg cells into effector cells [63]. The future investigation of Foxp3 expression in CD4⁺CD25⁺ Treg cells in cases of unexplained recurrent miscarriage, preeclampsia, etc appears, therefore, indicated.

Persistent toll like receptor (TLR) activation abrogates the immunoregulatory function of CD4⁺CD25⁺ Treg cells [52, 53]. It has been suggested that endometrial infections/inflammations may induce miscarriage [64]. Persistent TLR signals may reduce CD4⁺CD25⁺ Treg function, resulting in miscarriage. Excessive inflammation is observed in pre-eclampsia [64]. CD4⁺CD25⁺ Treg function may, indeed, be lost in pre-eclamptic cases since we observed that both, peripheral blood- and decidual-CD4⁺CD25^{high}, Treg cells decreased in pre-eclampsia [54]. Further studies are, of course, needed to better clarify normal numbers and function of CD4⁺CD25⁺ Treg cells in various complications of pregnancy.

Conclusion

Mice models reveal that alloantigen (paternal antigen)-specific T cells are selectively deleted during pregnancy, and paternal antigen-specific CD4⁺CD25⁺ Treg cells increase, playing an important role in successful pregnancy by preventing implantation failure (occult loss), spontaneous abortions, and pre-eclampsia. Murine trophoblast express MHC class I antigens, but human villous trophoblasts do not express either MHC class I or class II antigens. EVTs only express HLA-C, HLA-G and HLA-E in humans. Therefore, it has been unclear whether paternal antigen specific Treg cells are needed in the maintenance of pregnancy. We should clarify this simple point. In the meantime, pregnancy remains a rather mysterious phenomenon to immunologists.

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