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T Receptor Lymphocytes - Can they be Used as a Low Cost Predictive Tool for Dysfunctional Placental Diseases?

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Abstract

Background: Regulatory T cells (Tregs) are lymphocytes originating in the bone marrow. They mature in the thymus and help fight inflammation in humans. Tregs are involved in the maternal acceptance of the allogeneic fetus.

Objectives: This review focuses on the role of Tregs i.e. the origins, development and function in pregnancy, as well as their diagnostic and therapeutic value in pregnancy pathologies such as miscarriage and preeclampsia (PE).

Methods: A MeSH and text word PubMed search through August 2016 was performed using the keywords: T receptor lymphocytes; subsets of regulatory T cells; regulatory T cells; controlled trials; pregnancy and treatment. Additional databases searched included; Google scholar, database of abstracts of reviews of effects (DARE), Cochrane Library, NIHR central portfolio management system, UK database of uncertainties about the effects of treatments (DUETs), trip database, health on the NET Northern Ireland (HONNI), the knowledge network Scotland, the Geneva foundation for medical education and research (GFMER) and ClinicalTrials.gov.

Findings and conclusions: The origins, development, function and the role of Tregs in the pathophysiology of pregnancy and its complications have not been fully clarified. A lack of Treg cells or defective production due to placental disease or an altered immune response are found in women with sub-fertility, recurrent miscarriage, PE and preterm birth. However, clinical studies to date have involved small numbers of women (with a paucity of randomized controlled trials). Future research focusing on Tregs and their diagnostic and therapeutic value in the management of pregnancy complications are urgently needed.

Keywords: T receptor lymphocytes; Subsets of regulatory T cells; Regulatory T cells; Controlled trials; Pregnancy; Treatment

Introduction

The human immune system

The immune system helps to fight against infections as well as cellular mutations [1]. The immune system has 2 components, the innate and adaptive immune systems [1]. The innate immune system serves as the body's first line of defense, responding to microbes in a non-specific manner [1].

It employs NK cells (natural killer cells) that works to maintain self-tolerance and to eliminate viral infected host cells. Furthermore, it acts by communicating with small signaling proteins called cytokines [2-6]. The binding of a cytokine to a cell surface receptor starts an intra-cellular reaction chain that alters cell function to support or suppress inflammation [7-9]. Therefore, cytokines can be broadly divided into two groups; pro and anti-inflammatory [7-9].

Specific pro-inflammatory cytokines, including interleukin (IL)-1 and tumor necrosis factor-alpha (TNF- α) are stimulated by antibody release [10]. The activation of innate cells, including macrophages, neutrophils, dendritic cells and mast cells, induces the stimulation of other cytokines that support an inflammatory response such as IL-1, TNF- α , IL-4, IL-6 and IL-10 [10].

The adaptive immune system is the body's second line of defense, providing a complex and specialized response to infection [11,12]. The adaptive immune system is able to provide this type of response based on prior pathogenic exposure and elimination [11,12]. An adaptive immune response relies on both B lymphocytes/B cells and T lymphocytes/T cells [13]. B cells originate and mature in the bone marrow, and are fundamental to pathogen recognition [13]. Effector B cells circulate in the body until they encounter an antigen, which is then absorbed by it, and an antibody

specific for that antigen is secreted [12-15]. Some effector B cells differentiate into memory B cells, which have a higher binding affinity for the antigen [3].

T cells mature in the thymus, and prior to maturation, express both CD4 and CD8 receptors [16]. T cells then differentiate generally into either T helper cells (TH cells; CD4+ cells), which express the CD4 protein on the cell membrane, or cytotoxic T cells (Tc cells; CD8+ cells), which express the CD8 protein on its cell membrane [16]. **Figure 1** depicts the main parts of the human immune system in a very basic format.

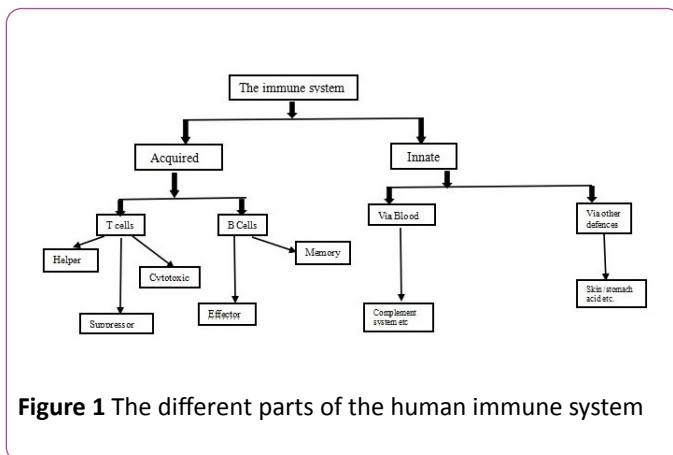


Figure 1 The different parts of the human immune system

Regulatory T cells (Tregs)

Regulatory T cells (Tregs) are lymphocytes that inhibit the function of other immune cells [17]. They originate in the bone marrow, mature in the thymus and are potent suppressors of inflammation [17]. Cytokines such as IL-2 play important roles in supporting the development of Tregs in the thymus, as well as their survival outside the thymus [18]. The maintenance of Treg cell function also requires the expression of the Foxp3 transcription factor [18]; mutation of the Foxp3 gene leads to Treg cell deficiency and autoimmunity in both mouse and humans [18,19].

Tregs are classified as follows

1. **Natural Tregs:** which are CD4+ CD25+ T cells that develop in the thymus where they are clonally selected and then move out of the thymus to perform their role [18,19].
2. **Adaptive Tregs:** are non-regulatory CD4+ T cells that attain CD25 expression outside of the thymus, typically, as a consequence of inflammation and disease processes. Adaptive Tregs mature in peripheral sites, including mucosa-associated lymphoid tissue (MALT) from CD4+ Treg precursors, as well as in the uterus in eutherian mammals [19].

Objective

This review focuses on the role of Tregs i.e. the origins, development and function in pregnancy, as well as their possible diagnostic and therapeutic value in pregnancy pathologies such as miscarriage and PE.

Methods

A MeSH and text word PubMed search through August 2016 was performed using the keywords: T receptor lymphocytes; regulatory T cells; subsets of regulatory T cells; controlled trials; pregnancy; and treatment. Additionally, we searched Google Scholar, database of abstracts of reviews of effects (DARE), Cochrane Library, NIHR central portfolio management system, UK database of uncertainties about the effects of treatments (DUETs), trip database, health on the NET Northern Ireland (HONNI), the knowledge network Scotland, the Geneva foundation for medical education and research (GFMER) and ClinicalTrials.gov. Most of the literature that was retrieved were observational and experimental studies mostly on animal models. There were no randomized trials or meta-analysis [20]. We have summarized our findings below.

Findings

Tregs in pregnancy

Medawar suggested that the fetus should be perceived as an allograft [21]. Pregnancy is unique as a semi-allogeneic conception is tolerated rather than rejected by the mother [22]. Immune disturbances have been linked to reproductive failure [22]. Thymic function is lower in women who are fertile and sex steroid hormones cause temporary thymic involution/atrophy during pregnancy [22]. Thus, the thymus is unlikely to be the source of the high numbers of peripheral and decidual Tregs found in pregnancy [20-22].

Non-specific circumstances, such as sub-optimal antigen presentation, and hormonal factors (elevated estrogen levels around ovulation and high progesterone concentrations in pregnancy), together with paternal allo-antigens, result in expansion of Tregs [20-22].

Tregs are required for the success of any pregnancy by suppressing immune mediated embryo rejection [23]. The literature implicating Tregs in the pathophysiology of pregnancy is expanding, but the immune basis for pregnancy complications such as preeclampsia remain poorly understood [4,24-27].

Elevations of specific cytokines have been inconsistently reported in preeclampsia, and the origins of such elevations are not known [28]. Cytokines are not invalid markers of immune function, but their transiency and susceptibility to environmental influences may significantly affect their quantification [28].

Tregs are particularly valuable for studying immunological defects during pregnancy, based on their stability and function during early pregnancy [29-32]. The physiological function of Tregs in pregnancy begins before implantation [32]. Treg numbers were found to be increased in several organs i.e. lymph nodes, blood, spleen and thymus, prior to implantation [33]. Expansion of Tregs have been observed in fertile non-pregnant women in the late follicular phase of the menstrual cycle [34]. This increase is associated with the rise in estradiol

levels [33]. It is thought that Tregs serve to prepare a non-hostile environment for the foreign paternal seminal fluid [35], and seminal fluid, in turn, can drive Treg expansion [33], thus preparing the endometrium to receive the embryo [36].

If implantation is successful, the body responds to the embryo as an allo-antigen, and a second wave of Treg recruitment and migration to the fetomaternal interface occurs [35,36]; Tregs accumulate in the decidua and suppresses an inflammatory rejection of the implanted embryo [9,37]. It is hypothesized that Treg migration is assisted by various proteins and receptors [38].

Human chorionic gonadotropin (hCG) produced by human trophoblast cells have been shown to facilitate migration of human Tregs along a hormonal gradient [33,39]. There is unanimous agreement that Treg migration occurs shortly after implantation [39-41]. The origin of these migrating Tregs is unclear; some reports suggest that Tregs travel directly from the thymus to the fetomaternal interface, while others indicate that this Treg migration is facilitated by the depletion of peripheral Tregs [40,42].

After implantation, the activity of Tregs is even less well studied [40,42]. Both human and murine models provide a consensus that decidual Tregs consistently expand in numbers throughout pregnancy [19,43,44]. Equally, studies have reported that peripheral Tregs expand in the first trimester, and either remain stable or continue to increase during the second and third trimesters [39,42]. Other studies have shown that peripheral Tregs are reduced in the second trimester [45], as well as in the third trimester, relative to the postpartum period [46], supporting the idea that upon implantation, circulating Tregs migrate to the fetomaternal interface during pregnancy [46].

Furthermore, the persistence of Tregs in the circulation suggests a degree of Treg memory to the paternal antigens [46], this could be a reason why PE and preterm birth are less frequent in the subsequent pregnancies [47].

A Foxp3 enhancer (in placental mammals), conserved non coding sequence 1 (CNS1), is pivotal to the generation of peripheral Tregs, but not Tregs of thymic origin [48]; this supports the accumulation of Tregs in the decidua, and a deficiency in CNS1 is thus postulated to lead to an increase in spontaneous miscarriages [48]. Foxp3 is a master gene for differentiation of Tregs [48]. Estrogen augments Foxp3 expression both *in vitro* and *in vivo*. Reduced endometrial Foxp3 mRNA may impair differentiation of uterine Tregs, resulting in implantation failure [48].

Th17 cells, which secrete IL-17 and an array of pro-inflammatory cytokines, play important roles in the host defense against infection, autoimmune and chronic inflammatory diseases [42,49]. In pregnancy, the immune system has to protect the mother against potential infections (Th17 cells), and concurrently, modulate the maternal immune response to the semi-allogeneic fetus [50]. The balance between Th17 cells and Tregs has been implicated in the pathophysiology of pregnancy complications such as miscarriage and PE [50-52].

Tregs and sub-fertility

There are a limited number of publications on sub-fertility and Tregs. Sub-fertility has been associated with decreased numbers of the Treg transcription factor, Foxp3, in endometrial tissues [41,53], as well as an increase in Th1 cytokines [54]. These studies suggest that a deficiency in Treg mediated immune tolerance may have a detrimental effect on fertility (Figure 2) [55].

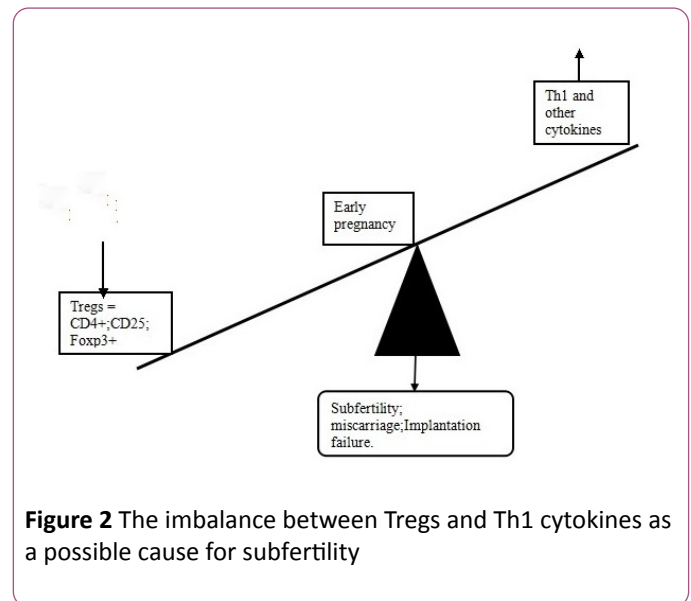


Figure 2 The imbalance between Tregs and Th1 cytokines as a possible cause for subfertility

Tregs and recurrent miscarriage

Recurrent miscarriage, defined as three or more spontaneous pregnancy losses before 20 weeks of gestation, occurs in 1% to 2% of reproductive couples, 60% of which are unexplained [56,57]. The prevalence of miscarriage following three or more miscarriages is 0.3% [56,57]. In addition to high number of NK cells, an increased population of CD4+ Th1 cells is also thought to be harmful in early pregnancy [51]. It has been suggested maternal immune rejection of the fetus occurs in unexplained recurrent miscarriage [27]. In relation to this, high Th17, high Th1, high NK and low Treg counts are associated with an increase in the incidence of unexplained recurrent miscarriage [51,58].

There are more studies on Tregs and recurrent miscarriage [51,58]. Similar to sub-fertility, the numbers of Tregs are low in recurrent miscarriage, specifically, it has been reported that decidual Treg levels are decreased in women prone to unexplained recurrent miscarriage [59], and low circulating Treg numbers can predict miscarriage [60].

Tregs and preeclampsia

Preeclampsia (PE) is a multisystem hypertensive pregnancy disorder affecting 2% to 8% of all pregnancies, and an important cause of maternal and neonatal morbidity and mortality [61,62]. The underlying cause of PE is not yet fully understood, however, impaired trophoblast invasion in early pregnancy could lead to PE and fetal growth restriction

[61,63]. **Figure 3** explains the correlation between Tregs and PE.

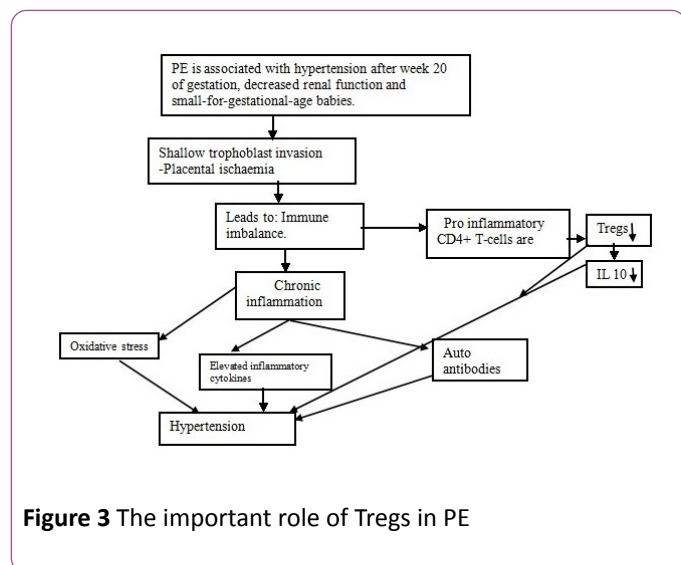


Figure 3 The important role of Tregs in PE

Derangements in the maternal tolerance to the fetus possibly as a result of perturbations in the Treg dynamics may play a role in the pathogenesis of PE and fetal growth restriction [61,63]. Some studies have described alterations in the adaptability of the immunological system and possibly its inappropriate activation can lead to a pro-inflammatory state and PE [61-65]. The pro-inflammatory state in PE could be explained by an increase in maternal Tc cells [64,65], and an increase in dendritic cell maturation [61].

With respect to Tregs, a research report has demonstrated that not only the numbers of Tregs are decreased but the function of Tregs are also impaired in preeclampsia [66]. Other researchers have shown, albeit in small numbers, lower peripheral and decidual Treg sub-types in pregnancies complicated with preeclampsia [67-72]. In addition, derangement in the differentiation of recent thymic emigrant (RTE) Tregs have been observed in women with PE [73,74].

How does a decrease in Treg numbers and activities cause PE? A well designed *in vitro* study showed that the treatment of decidual dendritic cells with trophoblast supernatant stimulated Transforming Growth Factor- β and consequent Treg proliferation, resulting in IL-10 production [75]. Further, this study found that Tregs significantly increased IL-10 mediated trophoblast invasion cells, mediated through IL-10 [75]. Decreased decidual Tregs can also lead to aberrant Th1 responses, of which TNF- α is a product [75]. TNF- α promotes the adhesion of lipoproteins to vascular walls, resulting in arterial thickening and increased blood pressure [76].

Finally, the balance between Tregs, Th2, Th17 and Th1 cells has been associated with PE [75-77]; a recent report demonstrated that whereas Th17 and Th1 cell numbers were unchanged, Treg and Th2 cell numbers were lower in the umbilical cord blood of women with PE [77].

Tregs and preterm birth

Preterm birth is defined as birth before 37 weeks of gestation, affecting approximately 11% of all live births, and associated with serious neonatal morbidity and mortality [78,79]. Immune cells contribute to a cytokine-rich milieu in the presence of infection and inflammation [78,79]. Pro-inflammatory cytokines such as TNF- α and IL-1 β stimulate the production of prostaglandins, which triggers a cascade of events leading to uterine contractions and fetal membrane rupture; if activated early, preterm labor can ensue [80].

It has been suggested that T cell recruitment to the fetomaternal interface is required for term pregnancy, and that the dysregulation of this process may lead to preterm labor and premature rupture of fetal membranes [81]. In relation to this, low circulating Tregs and short uterine cervical length is significantly associated with preterm labor [82]. The percentage of Tregs decreases in the decidua of pregnant women with spontaneous vaginal delivery compared to those with elective caesarean section without contractions, suggesting the possible role of Tregs not only in maintaining the pregnancy but also in the regulation of labor [83]. Furthermore, IL-6 concentrations are often elevated in preterm labor [84], which suggests that the Th17/Treg balance is altered in preterm labor [84]. Progesterone treatment can lower the risk of preterm labor [85]; notably, Tregs express progesterone receptors, indicating that the beneficial effects of progesterone could be through stimulating Treg proliferation [86].

Chronic chorioamnionitis exhibits a reaction immunologically similar to transplant rejection [20]. Moreover, Treg mediated suppression of the immune response can cause chronic low grade infection to persist in preterm pregnancies [87]. From a neonatal perspective, lower Treg activity may contribute to the inflammation in newborns associated with pregnancies complicated by chorioamnionitis [88]. On the other hand, the peripheral Treg pool of preterm infants may be altered by lower gestational age, prenatal exposure to inflammation and chorioamnionitis [89].

Treg therapy in pregnancy complications

Tregs can be isolated and expanded in *ex vivo* cultures, hence, a therapeutic product can be manufactured at relevant doses [33]. Intravenous administration and/or intrauterine injection of Tregs to raise Treg numbers at the fetomaternal interface appears to be an attractive option in the treatment of pregnancy complications such as miscarriage and preeclampsia. However, administration of Tregs to humans have been associated with serious immunological side effects [33]. Nevertheless, the therapeutic applications of Tregs to regulate immune responses remain an active area of research [90].

Conclusion

Reproductive complications such as sub-fertility, recurrent miscarriage, PE and preterm birth, pose a serious health, social

and economic burden on women and their families [90,91]. From our discussion above it has been seen that Tregs play an important part in normal pregnancy and various reproductive complications.

Understanding the mechanisms by which Treg cells exert their influence, is an area of intense research with broad implications for the development of therapeutic strategies for many disease processes including pregnancy related pathologies [90,91]. Tregs are involved in the maternal tolerance to the fetus at the feto-maternal interface [90,91].

The origins, development, function and the role of Tregs in the pathophysiology of pregnancy and its complications, however, have not been completely clarified [90,91]. A recent development is the identification of memory T cells that could function as Tregs, the clinical significance of which has yet to be fully defined [91].

Furthermore, we now understand that Tregs interact with NK cells to maintain immune balance in pregnancy [90,91]. Cell therapies with Tregs in early pregnancy may perhaps help to 'cure' imbalances in pregnancy complications [90,91].

A lack of Treg cells or defective production due to placental disease or an altered immune response are found in women with sub-fertility, recurrent miscarriage, PE and preterm birth [70-91]. However, clinical studies to date have involved small numbers of women (with a paucity of randomized controlled trials). Future research focusing on Tregs and their diagnostic and therapeutic value in the management of pregnancy complications are urgently needed.

Many authors propose that transferring antigen specific Tregs before implantation have worked in murine models and could work in humans as well [92-94]. Some innovative experiments have looked at vaginal application of TGB (tumor growth factor) that increases peripheral Tregs and halt miscarriages. A small ovine study by Willems et al. showed that systemic IL-2 treatment in utero, increased Tregs and improved lung gas volume in ovine fetuses with chorioamnionitis. However, these applications are still in the experimental stages. Furthermore, it is well known that animal testing is not always well replicated in humans and may be a waste of resources. Perhaps stem cell research may be the answer.

We thus concluded that it is still not safe to use Tregs for immune modulation in pregnancy. As the measurements of circulating Tregs become more accurate, stable and convenient, it may be a useful bio-marker for pregnancy complications [29-31]. Future research could be directed at quantification of specific Tregs in various different stages of complicated pregnancies. A cost benefit analysis could further enhance the usefulness of these trials.

At this present point, we are still a long way away, before we can safely use Tregs as a low cost diagnostic tool for dysfunctional placental disease.

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