

# Beyond Immune Balance: The Pivotal Role of Decidual Regulatory T Cells in Unexplained Recurrent Spontaneous Abortion

Qing-Hui Li<sup>1,2</sup>, Qiu-Yan Zhao<sup>1</sup>, Wei-Jing Yang<sup>1</sup>, Ai-Fang Jiang<sup>2</sup>, Chun-E Ren<sup>2</sup>, Yu-Han Meng<sup>2</sup>

<sup>1</sup>School of Clinical Medicine, Shandong Second Medical University, Weifang, Shandong, 261021, People's Republic of China; <sup>2</sup>Center of Reproductive Medicine, Affiliated Hospital of Shandong Second Medical University, Weifang, Shandong, 261000, People's Republic of China

Correspondence: Yu-Han Meng, Center of Reproductive Medicine, Affiliated Hospital of Shandong Second Medical University, No. 2428.Yuhe Road, Kuiwen District, Weifang, Shandong, 261031, People's Republic of China, Tel/Fax +86 536-3081389, Email yhmeng6@163.com

**Abstract:** Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy failures, which brings tremendous stress to women of childbearing age and seriously affects family well-being. However, the reason in about 50% of cases remains unknown and is defined as unexplained recurrent spontaneous abortion (URSA). The immunological perspective in URSA has attracted widespread attention in recent years. The embryo is regarded as a semi-allogeneic graft to the mother. A successful pregnancy requires transition to an immune environment conducive to embryo survival at the maternal–fetal interface. As an important member of regulatory immunity, regulatory T (Treg) cells play a key role in regulating immune tolerance at the maternal–fetal interface. This review will focus on the phenotypic plasticity and lineage stability of Treg cells to illustrate its relationship with URSA.

**Keywords:** immune homeostasis, Treg cells, phenotype, maternal–fetal tolerance

## Introduction

Immune self-stabilization is one of the three functions of human immune system.<sup>1</sup> Regulatory T (Treg) cells are named for their powerful function in regulating the immune system and improving immunological self-tolerance, which plays an important role in immune homeostasis.<sup>2,3</sup> Treg cells have been confirmed to be related to various immunological diseases such as rheumatoid arthritis (RA), type 1 diabetes, allergy and graft-versus-host disease (GVHD).<sup>4–7</sup> In recent years, substantial evidence has shown that Treg cells play a major role in fetal-maternal tolerance.<sup>8–10</sup> Treg cells not only suppress inflammation but also prevent the adverse effects of anti-fetal alloantigen, facilitating essential vascular adaptations crucial for placental morphogenesis at the maternal–fetal interface.<sup>11–14</sup> In the first trimester of human pregnancy, T cells comprise 10% to 20% of decidual immune cells,<sup>15</sup> of which Treg cells account for 10–30% of the CD4<sup>+</sup> T cells.<sup>16,17</sup> Phenotypic plasticity is an important characteristic of Treg cells, and lineage stability of Treg cells is crucial for their function. Since the 1970s, scholars have made efforts to characterize Treg cells by reliable molecular markers.<sup>18</sup> In the mid-1990s, Sakaguchi et al discovered that Treg cells constitutively and highly expressed CD25.<sup>19</sup> In 2003, transcription factor Fork head box P3 (FoxP3) was found specifically expressed in CD25<sup>+</sup>CD4<sup>+</sup> natural Treg cells in rodents and human,<sup>20–22</sup> which is a key determinant of their suppressive function. Subsequently, in order to better elucidate its function and heterogeneity, more and more markers have been found to characterize its phenotype, such as Helios, neuropilin-1 (Nrp-1), inducible co-stimulator (ICOS), programmed cell death protein 1 (PD-1), ITIM domain protein (TIGIT) and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), etc.<sup>23–27</sup> However, phenotypic plasticity and lineage stability of Treg cells is still a controversial topic.

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy failures according to the guidelines of the American Society of Reproductive Medicine.<sup>28</sup> A lot of evidence indicates that RSA is related to genetic defects, immune disorders, abnormal genital structure, specific and nonspecific inflammation, endocrine disorders and other factors.<sup>29–32</sup> The etiology of RSA is complex, and more than 50% of patients are unknown.<sup>33</sup> As the number of

miscarriages increases, the likelihood of URSA women experiencing early miscarriage, premature birth, placenta previa and other related complications also increase when they become pregnant again.<sup>34,35</sup> This can be serious adverse effects on women with URSA. In recent years, the immunological perspective in recurrent miscarriage (RM) has attracted widespread attention. Successful pregnancy requires the attachment of the embryo to the endometrium, decidualization of the endometrium, and the differentiation of blastocysts into trophoblasts to invade the decidua.<sup>10,36</sup> Abundant immune cells reside in the decidua in close contact with paternally derived alloantigens and fetal tissues. They participate in the establishing, sustaining and terminating pregnancy extensively.<sup>10</sup> As a member of regulatory immunity, the significance of Treg cells during the implantation and maintenance of the healthy pregnancy is evident. However, the role of Treg cells in URSA is still a topic worthy of further study. This review delves into the phenotypic plasticity and lineage stability of Treg cells and elucidates the relationship between Treg cell functions and URSA, aiming to present novel insights for immunological approaches to treating URSA.

## Classification of Treg Cells

According to differentiation, Treg cells can be categorized into two groups: natural Treg (nTreg) cells and induced Treg (iTreg) cells. During thymus development, immature T lymphocytes produce nTreg cells, identified by the presence of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells. Conversely, mature CD4<sup>+</sup>CD25<sup>-</sup> T cells can convert into iTreg cells under stimulation of peripheral antigen or induction of immunosuppressive factors. iTreg cells can be divided into Type 1 regulatory T (Tr1) and Th3 subsets. Tr1 Treg cells mainly produce interleukin (IL)-10, while Th3 cells primarily secrete transforming growth factor beta (TGF- $\beta$ ).<sup>4,37</sup>

In recent years, scholars have recommended that Treg cells can be classified into two categories according to their origins. The aforementioned nTreg cells may be termed thymus-derived Treg (tTreg) cells, originating from the thymus with a T-cell receptor (TCR) featuring relatively high self-affinity.<sup>38</sup> Periphery-derived Treg (pTreg) cells develop from CD4<sup>+</sup> effector cells under TCR signal transduction or other factors (such as TGF- $\beta$ , IL-2),<sup>39</sup> which mainly exist in peripheral barrier tissues and play an important role in controlling local inflammation.<sup>40</sup>

According to the function and location, Treg cells can also be divided into central Treg (cTreg) cells and effector Treg (eTreg) cells.<sup>41</sup> cTreg cells, expressing CC-chemokine receptor (CCR) 7 and L-Selectin (CD62L) at high levels, are mainly located in peripheral lymphoid tissue.<sup>42</sup> eTreg cells are mainly found in non-lymphoid tissues and can be identified by the presence of surface markers like ICOS or CD44.<sup>43</sup> They exhibit a remarkable ability to adapt and specialize to specific tissue environments.<sup>44,45</sup>

## Immunosuppressive Mechanism of Treg Cells

The immunosuppressive effect of Treg cells is primarily accomplished through the interaction of their inhibitory surface molecules with other immune cells. TIGIT presented on Treg cells interacts with CD155 on dendritic cells (DCs) to suppress the activation of effector T cells (Teffs), Th1, and Th17, which is achieved by IL-10 augmentation and IL-12 reduction.<sup>46-49</sup> Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) on Treg cells interacts with CD80 and CD86 on antigen-presenting cells (APCs), which results in the inhibition of antigen presentation and maturation functions of APCs.<sup>50</sup> Furthermore, the activation of indoleamine 2,3-dioxygenase (IDO) expressed in DCs ultimately leads to the suppression of Teffs.<sup>51</sup> PD-1 binds to its ligand PD-L1 and PD-L2 on DCs, which gives rise to the inhibition of Teffs via enhancing the transactivation of Smad3 by TGF- $\beta$ .<sup>52</sup> Binding of lymphocyte activation gene 3 (LAG-3) to major histocompatibility complex class II (MHC-II) molecules expressed on immature DCs induces inhibitory signaling pathways which suppresses DCs maturation and the activation of Teffs.<sup>53</sup> Caspase-8 activated by the combination of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and death receptor 5 (DR5) induces apoptosis in effector lymphocytes.<sup>54,55</sup> CD25, also known as interleukin IL-2 receptor (IL-2R), has been demonstrated to control the acquisition of cytotoxic activity of CD4<sup>+</sup>T cells by competing for IL-2.<sup>56</sup>

In addition to the molecules mentioned above, Treg cells exert their immunosuppressive functions through soluble intermediates. New evidence emphasizes the significance of adenosine and cAMP in the ability of Treg cells to inhibit Teffs.<sup>57,58</sup> Ectoenzymes CD39 and CD73 expressed on Treg cells were shown to raise the concentration of adenosine, which suppressed the function of Teffs through activating the adenosine A2A receptor.<sup>59,60</sup> Granzyme-A, granzyme-B

and perforin ensure the cytolysis of Treg cells to other immune cells, such as B cells, NK cells and CD8<sup>+</sup> T cells.<sup>61–64</sup> Anti-inflammatory cytokines, such as IL-10, TGF- $\beta$  and IL-35, mediate the anti-inflammatory effect of Treg cells.<sup>65</sup>

## The Relationship with Pregnancy

### The Origin of Decidual Treg Cells

Decidual Treg cells originate from peripheral blood Treg cells, including tTreg cells and pTreg cells, exhibiting varying phenotypic heterogeneity according to the cycle and environment.<sup>27,66,67</sup> Recruitment of Treg cells into the uterus commences in endometrial proliferation stage of each cycle and peaks at ovulation.<sup>68</sup> Estrogen in uterine and TGF- $\beta$  and prostaglandin (PGE) in seminal fluid play a role in recruiting macrophages and DCs, which makes them acquire M2 macrophages and tolerogenic DCs (tDCs) phenotypes. Interferon-gamma (IFN- $\gamma$ ) and IL-10 secreted by uterine natural killer (uNK) cells, Granulocyte-macrophage CSF (GM-CSF) and chemokines secreted by uterine epithelial cells also facilitate the acquirement of M2 macrophages and tDC phenotypes.<sup>69,70</sup> tDCs take up paternal alloantigens in seminal fluid and present antigen to Th0 cells in uterus-draining para-aortic lymph nodes (PALNs).<sup>71,72</sup> Later, Th0 cells can be activated and differentiated into pTreg cells. In mice, systemic expansion and accumulation of tTreg cells in the PALNs and uterus occur during the estrous stage in response to elevated levels of estradiol (E2) at ovulation.<sup>73</sup> During and before embryo implantation, pTreg and tTreg cells are recruited to the uterus and retained there. Treg cells increased in the early and middle trimesters and decreased prior to delivery, which is the similar pattern as Treg cells in peripheral blood.<sup>74</sup>

### Regulating Trophoblasts Invasion and Uterine Spiral Artery Remodeling

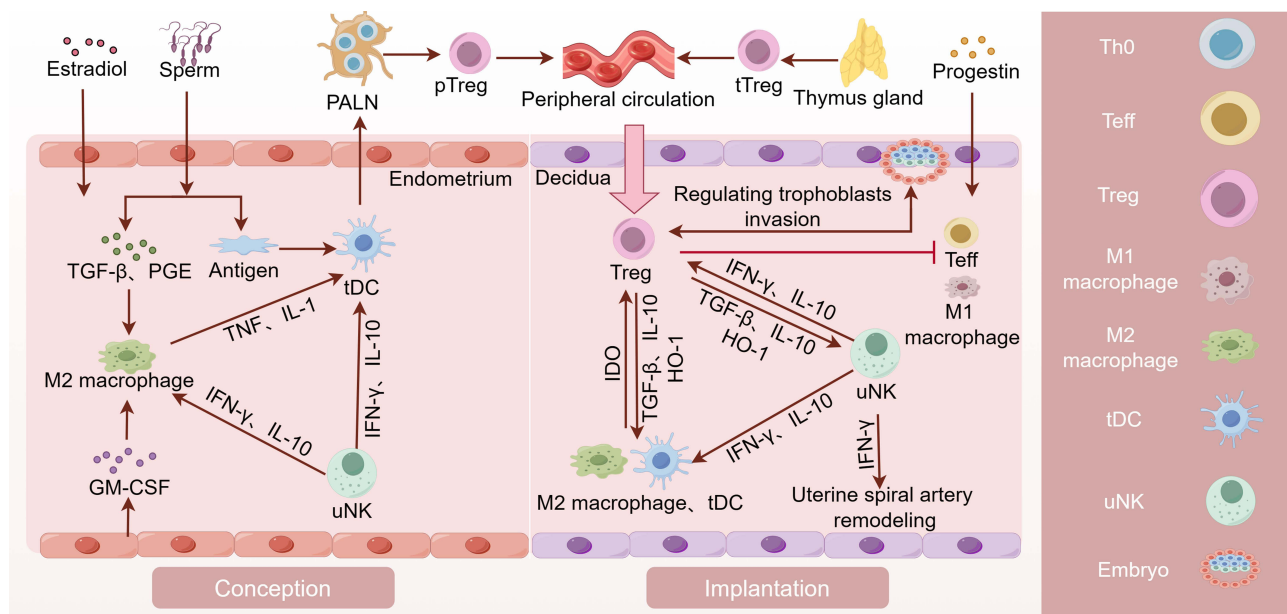
Embryo implantation necessitates trophoblast infiltration and remodeling of the uterine spiral arteries (SpA). During embryo implantation priming, the excessive production of IL-2 and IFN- $\gamma$  enhances the development of cytotoxic CD8<sup>+</sup> T cells, which subsequently contribute to fetal loss.<sup>75</sup> Meanwhile, unrestrained Teffs release inflammatory cytokines and play cytotoxicity effect on trophoblast through antigen-dependent, which adversely affects placental development.<sup>75,76</sup> Decidual Treg cells may contribute to constraining Teffs in early pregnancy by expressing CTLA4, CD25, and PD-L1, and secreting TGF- $\beta$  as well as IL-10.<sup>77,78</sup>

A variety of immune cells play a synergistic role in the embryo implantation, such as uNK cells, uterine dendritic cells (uDC) and uterine mast cells (uMC).<sup>79–81</sup> Treg cells cooperate with them to support the formation of decidua and promote embryo implantation.<sup>59</sup> M2 macrophages, tDC and uNK cells promote the peripheral differentiation of Treg cells and recruitment to the uterus.<sup>69,70</sup> On the one hand, Treg cells respond to epithelial cell-derived chemokine C-C motif ligand (CCL) 3, CCL4, CCL5, and CCL19.<sup>82,83</sup> Meanwhile, they inhibit the activation and function of Th1 and Th17 cells by consuming IL-2 or other inhibitory mechanisms.<sup>77,78,84</sup> On the other hand, Treg cells control inflammation by releasing TGF- $\beta$ , IL-10 and heme oxygenase-1 (HO-1) to interact with DCs and uNK cells.<sup>77,78,85</sup> This eventually facilitated decidualization and embryo implantation.<sup>51,85–88</sup> Besides, the regulatory loop between trophoblasts and maternal immune cell subsets might be bidirectional. An interesting finding suggests that trophoblasts regulated the differentiation of maternal CD4<sup>+</sup>T cells into immunosuppressive Treg cells, while CD4<sup>+</sup>T cells might promote the growth and invasiveness of trophoblasts.<sup>89</sup>

In recent years, increasing evidence has shown that Treg cells play an important role in the vascular endothelium and blood flow homeostasis.<sup>13,14</sup> uNK cells regulate the invasion of extravillous trophoblasts as well as displacement of endothelial cells and smooth muscle cells (SMCs) by releasing IFN- $\gamma$ , which ultimately facilitates the decidual vascular remodeling.<sup>81,90,91</sup> Treg cells suppress inflammatory activation and modulate the phenotypes of decidual uNK cells, macrophages and DCs by releasing TGF- $\beta$ , IL-10 and HO-1, which promotes the decidual vascular remodeling.<sup>16,77,78,85</sup> Treg cells restrict the activation and infiltration of M1 macrophages, so as to reduce the release of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and improve the vascular endothelial microenvironment.<sup>13,14</sup> At the same time, they inhibit the erosive effect of Th1 and Th17 on blood vessels,<sup>12,78</sup> which gives rise to diminished vascular resistance and increased blood supply to the placenta (Figure 1).

### The Phenotypes of Decidual Treg Cells

The Treg cell population during pregnancy exhibits remarkable diversity, both in the peripheral blood and at the maternal–fetal interface.<sup>9</sup> Until now, the populations of Treg cells at the maternal–fetal interface and their contribution



**Figure 1** Mechanisms of Treg Cells in Female Pregnancy. Estrogen and semen recruit macrophages and DCs, which promotes their polarization towards M2 macrophages and tDC phenotypes. tDCs uptake paternal antigens in semen and transport them to the PALN draining the uterus. Under the stimulation of paternal antigens, Th0 cells in PALN differentiate into pTreg cells. pTreg cells and tTreg cells converge in the peripheral circulation and re-enter the uterine cavity during the implantation stage to exert their functions. Decidual Treg cells restrict Teffs by secreting IL-10 and TGF- $\beta$  and expressing CD25, CTLA4 and PD-L1, which directly promotes the embryo implantation. Additionally, they work in collaboration with decidual immune cells to promote decidualization and enhance endometrial receptivity. Treg cells not only directly inhibit Teffs and M1 macrophages, but also work in collaboration with M2 macrophages, uNK, and tDC cells to promote trophoblast invasion and vascular remodeling. PALN, para-aortic lymph node. (By Figdraw).

to the decidual microenvironment have not been fully defined. Originally, a population of CD4<sup>+</sup>CD25<sup>+</sup> T cells, expressing intracellular CTLA-4, glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) and OX40 (CD134), was identified in the human decidua.<sup>92</sup> It is implied that Treg cells exist in decidua and play an important role in the regulation of local maternal tolerance towards the fetus.<sup>92</sup> Later, Dimova firstly demonstrated the presence and in situ distribution of CD4<sup>+</sup> Foxp3<sup>+</sup> cells in decidua,<sup>93</sup> which were classified into CD4<sup>+</sup> CD25<sup>++</sup> Foxp3<sup>+</sup>, CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup>, and CD4<sup>+</sup> CD25<sup>-</sup> Foxp3<sup>+</sup> subpopulations on the basis of CD25 expression.<sup>93</sup> These Foxp3<sup>+</sup> cells were found to express TGF $\beta$ 1 mRNA and exhibit surface consistent with Treg phenotype, including CD45RO (a marker for memory lymphocytes), CTLA-4, CD103, Nrp-1, LAG-3, and CD62L.<sup>93</sup>

Recently, three distinct types of decidual CD4<sup>+</sup> Treg cells in healthy pregnancies were investigated, whose phenotypes are CD25<sup>HI</sup> FoxP3<sup>+</sup>, PD1<sup>HI</sup> FoxP3<sup>-</sup> IL-10<sup>+</sup> and TIGIT<sup>+</sup> FoxP3<sup>dim</sup> Treg cells.<sup>94</sup> The characteristics of these three types of Treg cells are summarized in Table 1. In comparison to Treg cells in the blood, CD25<sup>HI</sup>FoxP3<sup>+</sup> Treg cells in the decidual tissue exhibit higher levels of co-inhibitory proteins or mRNAs, such as CTLA-4, GITR, CD39, ST2, and LRR32.<sup>94</sup> This observation indicates an enhanced activation and suppressive function of Treg cells in the decidual tissue, which plays a crucial role in regulating inflammation at the maternal–fetal interface.

Unlike the studies above, a systematic review discussed the heterogeneity within the FoxP3<sup>-</sup> Treg cell compartment and their role in pregnancy.<sup>9</sup> FoxP3<sup>-</sup>HLA-G<sup>+</sup>Treg cells have been identified in both decidua and peripheral blood during pregnancy, which plays a crucial role in establishing a tolerogenic decidual microenvironment by secreting IL-10 and soluble HLA-G.<sup>95–97</sup> Tr1 Treg cells, which primarily product IL-10<sup>98–100</sup> and express co-signaling molecules such as PD-1, CTLA-4, TIM-3, ICOS, GARP (LRR32), and latency associated peptide (LAP),<sup>101–103</sup> are also found in both peripheral blood and human decidua.<sup>94,104</sup> Additionally, the observation of mRNA cytokine profiles similar to Th3 represents the first description of a potential presence of Th3 cells in the decidua.<sup>93</sup> The main suppressive effects of Th3 Treg cells are mediated by TGF- $\beta$  in a cell-contact independent manner<sup>105,106</sup> (Table 1).

**Table 1** Different Phenotypes of Treg Cells During Pregnancy

Phenotypes	Characteristics	Function	References
CD25 <sup>Hi</sup> FoxP3 <sup>+</sup>	High expression of CD25, FoxP3 and Helios; the lack of CD45RA and CD127	Production of the lowest level of IL-10, IFN- $\gamma$ and IL-2; suppression of IFN- $\gamma$ and TNF- $\alpha$ secreted by CD4 <sup>+</sup> and CD8 <sup>+</sup> Teffs	[90]
PD1 <sup>Hi</sup> FoxP3 <sup>+</sup> IL-10 <sup>+</sup>	High expression of PD-1; the lack of FoxP3 and Helios; low CD25	Generation of the highest level of IL-10 and IFN- $\gamma$ ; suppression of proliferation of CD4 <sup>+</sup> (but not CD8 <sup>+</sup> ) Teffs in an IL-10-dependent manner	[90]
TIGIT <sup>+</sup> FoxP3 <sup>dim</sup>	High levels of TIGIT; low levels of FoxP3, Helios, PD-1 and CD25	Expression of the high levels of IFN- $\gamma$ and IL-2 and low levels of IL-10; inhibition of CD4 <sup>+</sup> Teffs proliferation	[90]
FoxP3 <sup>-</sup> HLA-G <sup>+</sup>	Secretion of sHLA-G and IL-10; cell interaction with HLA-G	The reduced killing capacity of T cells and NK cells; induction of the tolerant macrophages and DC cells	[91–93]
Tr1	Express CD49b, LAG-3, PD-1, CTLA-4, TIM-3, ICOS, GARP and LAP; produce IL-10 and TGF- $\beta$ ; low levels of IFN- $\gamma$ , IL-5, IL-2, and granzyme B; KIR receptors, ectoenzymes CD39 and CD73	Suppression of T cell proliferation and in favor of creating the tolerogenic decidual microenvironment; inhibition of other immune cells (such as DC and M $\phi$ )	[90,94–100]
Th3	Express Helios, LAP and GARP; secretion of TGF- $\beta$ and IL-10	Induction of DC-10s and Treg cells by IL-10; inhibition of NK cells and T cells and APC by TGF- $\beta$	[89,101,102]

**Abbreviations:** FoxP3, Forkhead box P3; IL-2,10, Interleukin-2,10; IFN- $\gamma$ , Interferon-gamma; TNF- $\alpha$ , The TNF-related apoptosis-inducing ligand; PD-1, Programmed death-1 ligand; Teffs, Effector T cells; TIGIT, The ITIM domain protein; HLA-G, Human Leukocyte Antigen G; sHLA-G, soluble Human Leukocyte Antigen G; NK, natural killer; DC, Dendritic; M $\phi$ , macrophages; APC, Antigen-presenting cell, TGF- $\beta$ , Transforming growth factor beta; LAG-3, Lymphocyte activation gene 3; CTLA-4, Cytotoxic T lymphocyte associated antigen-4, TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; ICOS, Inducible co-stimulator; LAP, Latency associated peptide.

## The Relationship with URSA

CD4<sup>+</sup> CD25<sup>+</sup> Treg-deficient mouse model was established to demonstrate that allogeneic fetuses are consistently rejected without Treg cells.<sup>107</sup> Transferring of Treg cells into mice prone to miscarriage resulted in an increase number of uMC, which possess a positive impact on the remodeling of the placenta and SpA.<sup>108</sup> Both in the peripheral blood and decidua, the number and function of Treg cells are diminished in women experiencing recurrent pregnancy loss (RPL), when compared to women in control group.<sup>109</sup> This evidence confirmed that insufficient Treg numbers or inadequate function were implicated in RSA.<sup>10</sup> The following is the main hypothesis of the relationship between Treg cells and URSA.

## Systemic Immune Imbalance

A number of scholars have analyzed the endometrial cytokine profile in normal female and patients with recurrent abortion. The luteal-phase endometrium of patients with URSA exhibited increased expression of the inflammatory mediators, such as TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$ .<sup>110,111</sup> At the same time, the levels of TGF- $\beta$ , IL-4, IL-10, leukemia inhibitory factor (LIF), and vascular endothelial growth factor (VEGF) were reduced in the endometrium of URSA patients during the luteal phase.<sup>110,111</sup> In addition, several studies demonstrated that the endometrium of women with URSA was accompanied by the alteration of uNK cells and reduced expression of key angiogenic regulators.<sup>112–115</sup> TGF- $\beta$  and IL-10 secreted by Treg cells were not only involved in immune regulation but also directly benefited the vasculature at the maternal–fetal interface.<sup>116</sup> The increased level of TGF- $\beta$ 1 in serum may lead to a reduction in the abortion rate in a mouse model prone to miscarriage.<sup>117</sup> Overall, this evidence indicates that the embryo may not survive in the inflammatory stage of implantation due to the failure to transition to an anti-inflammatory and proangiogenic immune environment. These dysfunctions may be associated with reduced numbers of Treg cells and the increase of Th17, Th1 and M1 macrophages in decidua and peripheral blood.<sup>118–123</sup>

## Phenotypic Plasticity and Lineage Stability

Cytokines, hormones, micro-RNAs, the reproductive tract microbiome, and seminal fluid composition all have the potential to interfere with the response of Treg cells,<sup>124–127</sup> because the newly generated pTreg cells are susceptible to lineage instability and phenotype switching.<sup>128</sup> CD4<sup>+</sup> T cells from patients with RM were cultured with DCs and the partner's seminal fluid antigens, which suggested that CD4<sup>+</sup>IL-17<sup>+</sup> and CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells proliferated excessively.<sup>129</sup> At



the same time, the study revealed that fewer CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Treg cells were generated by patients with RM compared with fertile controls.<sup>129</sup> High levels of IFN- $\gamma$  could skew Th0 differentiation toward Th17 cells and caused Treg cells to transdifferentiate.<sup>130,131</sup> In addition, deficiency in IL-10 triggers an unstable Treg response in the decidua, resulting in a quicker conversion of phenotypes and impairing ability to effectively anti-inflammation during the later stages of pregnancy.<sup>132,133</sup> There remains a highly contentious topic: pTreg cells shift the phenotypes and express cytokines that are characteristic of Teff lineages within hyperinflammatory environment.<sup>128,131</sup> Treg cells undergoing trans-differentiation into Th1 or Th17 cells were known as exTreg cells, which promote inflammation and other immune responses.<sup>128</sup> The phenotypic plasticity and switching abilities of Treg cells may contribute to the maternal ability to invest differently in reproductive opportunities. In normal circumstances, Treg cells maximize the maternal decidual receptivity and offspring adaptability by interacting with the environment, hormones, and cells.<sup>126,128,134</sup> However, there is also study showing that excessive immune adaptation during pregnancy might predispose pregnant females to the susceptibility of viral infections.<sup>135</sup> The ability of Treg cells to transdifferentiate into Teffs under conditions of severe infection, excessive inflammation, or disruption of fetal development allows for the termination of pregnancy to preserve maternal survival.<sup>136</sup>

Recently, evidence has focused on immune checkpoint molecules and stability markers of Treg cells, such as Foxp3, Helios, Nrp-1, ICOS, PD-1, TIGIT and TIM-3, which is aimed to explain the stability of inhibitory function of Treg cells.<sup>23–27</sup> The signature of endometrial Treg transcriptomic was identified.<sup>118</sup> The researchers observed increased expression of sphingosine-1-phosphate receptor 1 (S1PR1) and decreased levels of TIGIT protein in women with primary URSA, which suggested reduced inhibitory capacity of Treg cells in women with primary URSA.<sup>118</sup> In addition, Hu et al discovered that Tim3<sup>+</sup> Treg cells constituted over 60% Treg cells in the mouse decidua during early pregnancy.<sup>8</sup> Meanwhile, they observed the significantly lower Tim-3 expression on Treg cells in URSA, indicating that decreased Tim-3<sup>+</sup> Treg cells might have a close relationship with impaired immunologic tolerance in women suffering URSA.<sup>8</sup> In addition, Treg cells from women with RM exhibited fewer CD45RA<sup>-</sup> cells and reduced expression of CTLA4 and Ubc13(an ubiquitin E2 conjugating enzyme),<sup>66</sup> which implied that the stability of Treg cells was reduced in URSA<sup>120,129,137</sup> (Figure 2).

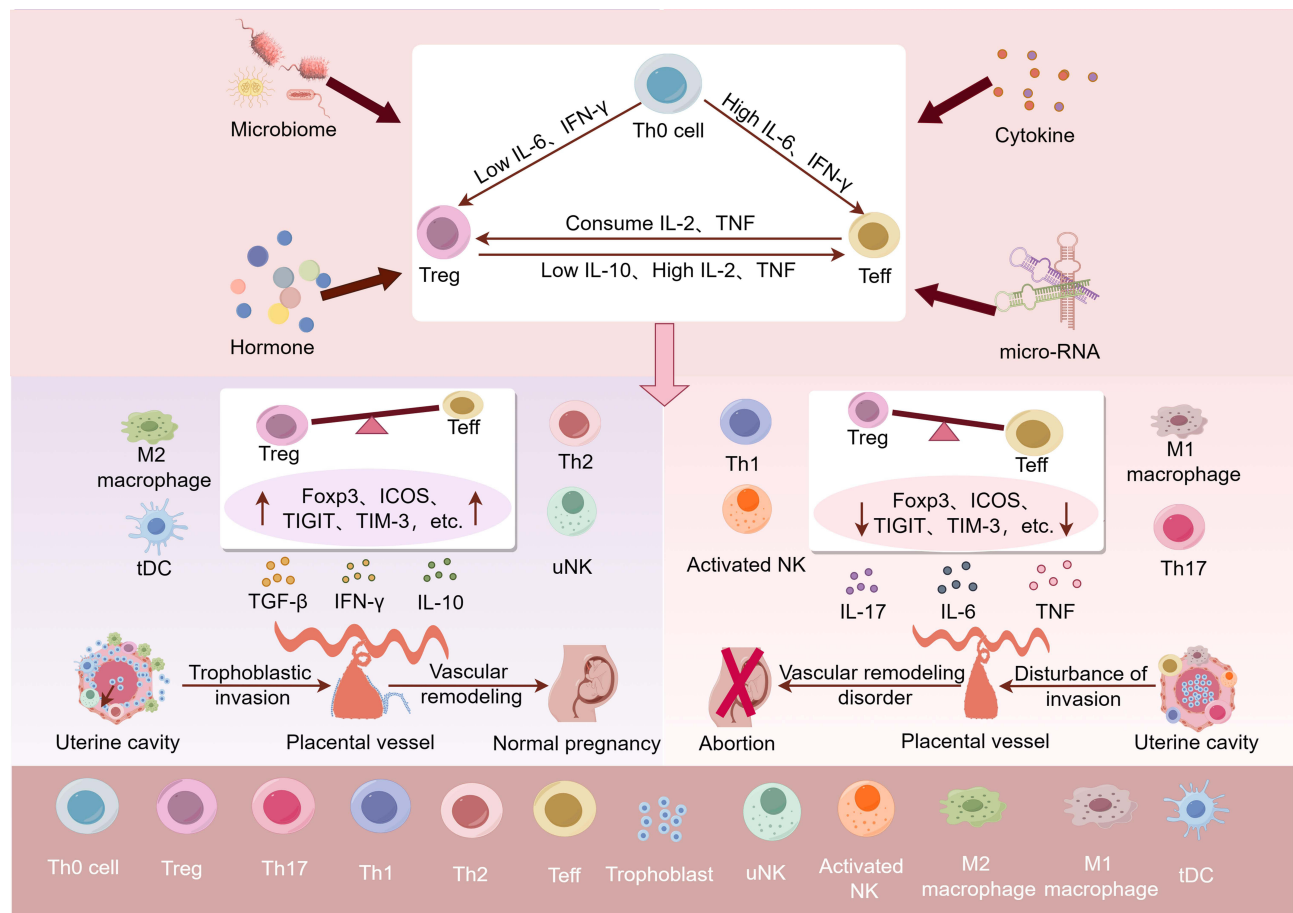
## Therapeutic Prospects for Targeting Treg Cells

Reproductive disorders caused by instability or insufficient production of Treg cells pose a challenging problem to be addressed, which may be the result of multiple factors in the biological evolution process.<sup>27,66,134,138</sup> Interventions aimed at increasing the number and improving the function of Treg cells are currently being developed and are showing promise in the treatment of tissue transplantation and autoimmune diseases. With the rapid progress in Treg cell therapeutics, there is a great potential for targeting Treg cells to address URSA.<sup>139</sup> Here, we propose three points:

### Signaling of Treg Cells and Stability

In recent years, some scientists have proposed promoting the stability of Treg cells through selective gene knockout. However, the safety concerns associated with this approach must be taken into consideration. For example, IL-6 triggers the signal transducer and activator of transcription 3 (STAT3) transduction pathway, which then induces the expression of the DNA methyltransferase 1 (DNMT1).<sup>140,141</sup> This leads to the methylation and subsequent downregulation of the Foxp3 site, ultimately resulting in the development of naive T cells into Th17 cells.<sup>142</sup> Therefore, targeting IL-6 receptor (IL-6R) or STAT3 in Treg cells could be a viable strategy to enhance the stability of Treg cells and protect them from alterations caused by IL-6 signaling.<sup>143</sup> In human, IL-6R-targeted antibodies have been identified as a potential therapeutic approach for inflammatory and autoimmune diseases like RA, Crohn's disease, and systemic lupus erythematosus (SLE).<sup>144–146</sup>

IL-2 plays a crucial role in the generation, survival, stability, and function of Treg cells.<sup>147</sup> In the absence of IL-2, Treg cells undergo apoptotic death, which leads to autoimmunity.<sup>148</sup> Therefore, various strategies have been developed to utilize IL-2 as a therapeutic pathway to improve the stability, effectiveness and survival of Treg cells in vivo.<sup>147,149</sup> The hypothesis suggests that administering low doses of IL-2 would primarily activate Treg cells and restrict the activation of Teffs, which is contrary to the impact of high doses of IL-2.<sup>150–153</sup> Two therapeutic strategies targeting IL-2 have primarily been developed, and IL-2 low-dose therapy and monoclonal antibodies that target IL-2. F5111.2, a fully human



**Figure 2** The relationship between Decidual Treg cells and URSA. In human, cytokines, hormones, micro-RNAs, the reproductive tract microbiome, and seminal fluid composition all have the potential to interfere with the immune balance at the maternal–fetal interface. High levels of IL-6 and IFN- $\gamma$  could skew Th0 differentiation toward Teffs and cause Treg cells to transdifferentiate. Newly generated pTreg cells are susceptible to lineage instability and phenotype switching. High levels of IL-2, TNF and low levels of IL-10 could skew pTreg cells differentiation toward Teffs. If pTreg cells express additional markers associated with functional stability, such as Foxp3, ICOS, TIGIT, and TIM3, it could potentially shift the immune balance at the maternal–fetal interface towards immune tolerance. This may lead to elevated TGF- $\beta$ , IL-10, M2 macrophages and tDC cells, which promotes trophoblast invasion, vascular remodeling and placental development. Conversely, if the newly generated pTreg cells are unstable, it could shift the immune balance at the maternal–fetal interface towards pro-inflammation. This could result in elevated IL-6, IL-17, TNF, M1 macrophages, Th1 cells and etc, which may hinder trophoblast invasion, vascular remodeling, and ultimately causing placental dysplasia and URSA. (By Figdraw).

anti-IL-2 antibody, were recently developed to induce the preferential expansion of human Treg cells by blocking IL-2R $\beta$  and reducing IL-2R $\alpha$ .<sup>152</sup>

## Interventions to Increase Treg Cell Numbers

The decrease in number of Treg cells in the decidua of women with URSA is likely a consequence of impaired generation or recruitment of Treg cells during pregnancy establishment,<sup>118,154</sup> which suggests that Treg cells possess therapeutic potential in women with URSA.<sup>155</sup> Co-expression of a chemokine receptor, which could identify chemokines in an inflammatory environment, may improve Treg cell functionality.<sup>143</sup> In related studies, overexpression of chemokine receptors has been found to improve chimeric antigen receptor (CAR) T cells homing to the tumor, resulting in enhanced antitumor activity and improved survival.<sup>156–158</sup> A recent study elucidated that histone methyltransferase Nsd2 upregulated the expression of C-X-C chemokine receptor type 4 (CXCR4) through the H3K36me2 modification, which plays a crucial role in promoting the recruitment of Treg cells into the decidua and ultimately improved pregnancy outcomes in mice.<sup>159</sup>

In addition to the methods of increasing number of Treg cells endogenously, methods of artificially supplementing Treg cells have been developed. The method of adoptive transplantation of Treg cells to improve the prognosis of spontaneous abortion has demonstrated effectiveness in mouse models and is anticipated to be a promising immunological treatment for women with URSA.<sup>155</sup> Evidence suggests that Treg cells from umbilical cord blood display higher repertoire diversity and

lineage stability compared to those from adult peripheral blood, providing a feasible basis for Treg cell therapy.<sup>160</sup> Before the development of endogenous Treg cells from a donor's bone marrow cells, using cord blood-derived Treg cells could offer a temporary solution.<sup>143</sup>

## Behavioral and Pharmacological Interventions in URSA

Metabolic, autoimmune conditions, inflammatory exposures and age strongly affect the immune response.<sup>139,161–163</sup> Metabolic imbalance, such as hyperglycemia and insulin resistance, will skew the energy source driving the T cell pool, which eventually results in an increased number of Th17 cells and declined in the number of Treg cells.<sup>164</sup> Microbiome disorders, deficiencies in micronutrients and vitamins have a specific impact on Treg cells. Treating these disorders and deficiencies is anticipated to enhance uterine immune function,<sup>165</sup> which may improve the prognosis of URSA in turn.

Besides, preexisting health conditions and lifestyle factors are also significant in male partners, because of the affection of seminal fluid quality and healthy female response.<sup>166</sup> Incompatibility or insufficient disparity of HLA between partners may lead to low immunogenicity of male alloantigens, leading to either excessive inhibition or activation of uNK cells or hindering the priming and expansion of the Treg cell population.<sup>167</sup> Providing guidance on seminal fluid priming during preconception planning may be a promising approach for nulliparous women without known compatibility issues.<sup>167</sup> More interestingly, exercise and sunlight exposure potentially increase Treg cells by regulating their homeostasis.<sup>168,169</sup> Intravenous immunoglobulin, prednisolone, and TNF inhibitors, initially developed for other autoinflammatory or specific autoimmune diseases,<sup>61,170–172</sup> have been investigated in patients with URSA and recurrent implantation failure. However, the clinical data supporting efficacy are limited.<sup>173</sup> Progesterone has been shown to effectively inhibit the generation of Th1 and Th17 cells while also inducing Treg cell differentiation.<sup>61,174–176</sup>

## Conclusion and Prospects

The combination of animal models and clinical studies provide evidence that decidual Treg cells play a role in reducing inflammation during the early pregnancy. They also contribute to creating an environment in the decidua that supports implantation receptivity and placenta formation. Immune maladaptation or imbalance leading to instability or insufficient of Treg cells, which may be a cause of URSA. A large number of studies should be devoted to investigate the subsets of Treg cells, which can gain more insights into their functions and roles in URSA. It is necessary to continually explore ways to improve the number and stability of Treg cells, which may be a therapeutic target for URSA.

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## Disclosure

The authors report no conflicts of interest in this work.

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