



# The Role of Immune Cells in Recurrent Spontaneous Abortion

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## Abstract

Recurrent spontaneous abortion affects approximately 1–2% of women of childbearing, and describes a condition in which women suffer from three or more continuous spontaneous miscarriages. However, the origin of recurrent spontaneous abortion (RSA) remains unknown, preventing effective treatment and placing stress upon patients. It has been acknowledged that successful pregnancy necessitates balanced immune responses. Therefore, immunological aberrancy may be considered a root cause of poor pregnancy outcomes. Considerable published studies have investigated the relationship between various immune cells and RSA. Here, we review current knowledge on this area, and discuss the five main categories of immune cells involved in RSA; these include innate lymphocytes (ILC), macrophages, decidual dendritic cells (DCs), and T cells. Furthermore, we sought to summarize the impact of the multiple interactions of various immune cells on the emergence of RSA. A good understanding of pregnancy-induced immunological alterations could reveal new therapeutic strategies for favorable pregnancy outcomes.

**Keywords** Recurrent spontaneous abortion · Maternal-fetal immunity · Immune cells · Immune dysfunction

## Introduction

Recurrent spontaneous abortion (RSA) is a serious reproductive disorder of pregnancy that presents an unresolved issue in the fields of gynecology and obstetrics. In China, RSA is usually defined as a woman suffering from  $\geq 3$  spontaneous abortions with the same sexual partner. The incidence rate of RSA ranges from 1 to 5% in women of childbearing age [1]. Recent research has determined that the etiology of RSA is extremely varied, chiefly advanced high maternal age, inheritable genetic abnormalities, anatomical factors, infections, and endocrine dysfunctions [2]. However, in most patients, the cause is unclear. Immune dysfunction accounts for more than half of these cases [3], and is usually referred to as immune-related RSA [4]. A successful pregnancy requires an accurate immunologic dialogue at the maternal-fetal immune interface in the endometrium. During early gestation,

the occurrence of immunologic events over bilateral communication between the mother and fetus is extremely elaborate, and encompasses a great deal of immunocytes, including innate lymphocytes (ILC), macrophages, decidual dendritic cells (DCs), and T cells. These cells play a crucial role in establishing a balance between the inflammatory response and immune tolerance. Existing evidence indicates that disorders occurring in the endometrial immune microenvironment are related to severe crucial reproductive disorders, which involve recurrent implantation failure (RIF) and RSA with inexplicable etiology [5]. In order to profile several immune cells included in RSA pathogenesis and recognize potential alloimmune risk factors, this review summarizes current research on the immune mechanism of RSA to expand our knowledge of its immune etiologies. However, in this article, we did not take into account B lymphocytes, since it may be closely related to antiphospholipid syndrome known as a cause of RSA [6, 7].

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## Innate Lymphoid Cells

Innate lymphoid cells (ILCs) play significant roles in membrane immunity, tissue equilibrium, and metabolism regulation, and have inspired much research in recent years [8]. Researchers have identified that ILCs exist in the human

decidua and are crucial at the maternal-fetal interface [9]. ILCs with short antigen receptors substantiate a classical lymphoid cell morphology, depending on two important components (cytokine receptor  $\gamma$ -chain and the transcriptional repressor inhibitor of DNA binding 2) [10, 11]. Based on distinct developmental pathways, they are classified into two subfields: natural killer (NK) cells and non-cytotoxic helper ILCs, including ILC1s, ILC2s, and ILC3s [12]. Decidual natural killer (dNK) cells are the only subset of ILCs with cytotoxicity. Maladjustment of cytotoxic regulation transforms dNK cells into harmful cells and leads to reproductive disorders, including RSA [13, 14]. Its internal mechanisms have been extensively researched, including the unbalanced expression of activating and inhibiting receptors on the surface of dNK cells. This includes the increased presentation of NKG2D and the lack of KIR, the combination of which causes adverse pregnancy outcomes [15, 16]. Furthermore, it has been found that in the mouse uterus, dNK cell cytotoxicity is usually altered by the unbalanced expression between Tumor Necrosis Factor-Like Weak Inducer of Apoptosis TWEAK and its receptors, which could result in abortion [17]. More than half of the women who experience RSA are diagnosed with unexplained recurrent pregnancy loss [18]. Some of them may suffer immune defects, such as disorganized NK cells or abnormal NK cell subpopulations [19]. The quantity and viability of peripheral blood NK (pNK) cells may also play an important role in RSA development. Women who suffer from RSA generally have higher active performance in the quantity and viability of pNK cells compared with normal pregnant women [20, 21]. This may be related to the high cytotoxic activity of pNK [22]. Human NK cells are categorized into four types, including NK1, NK2, NK3, and NKr1 subsets, based on cytokine production [23]. Among them, NK1 produces IFN- $\gamma$  and TNF- $\alpha$ , and NK2 excretes IL-4, IL-5, and IL-13. NK3 cells produce TGF- $\beta$ ; and NKr1 cells produce IL-10 [24]. In order to achieve a good pregnancy outcome, NK cells may change from type 1 to type 2 immune responses [25]. A previous study presented an obvious type 1 shift in pNK cells in patients with recurrent implantation failure (RIF) or RSA [26]. This suggests that the growth in the NK1/NK2 ratio may be an indicator for the probability of pregnancy failure. In addition, CD56<sup>bright</sup>/CD16 accounts for almost 90% of uterine NK (uNK) cells [27]. Cells with low cytotoxic activity produce more cytokines. Women with RSA had a smaller number of uNK than fertile controls, indicating that recurrent spontaneous abortion is closely associated with an abnormal proportion of uNK cells [28]. It is well known that uNK cells are indispensable for controlling trophoblast invasion and proliferation [29].

Successful pregnancy depends on correct spiral artery remodeling. During the process of placentation, the purpose of invasive extravillous trophoblasts (EVTs) moving to the uterus is to remodel vessels. Spiral artery remodeling by EVT

plays an important role in adapting blood flow and delivering nutrients to developing fetuses [30]. Impaired spiral artery remodeling has been linked to early miscarriage [31]. uNK cells are the major source of various cytokines, including GM-CSF, CSF-1, TNF- $\alpha$ , IFN- $\gamma$ , TGF- $\beta$  [23], and angiogenic growth factors. A previous study has shown that in women with RSA, the expression profile of angiogenic factors in CD56<sup>bright</sup> uNK cells displays a significant overexpression of angiogenin, bFGF, and VEGF-A. This may be relevant to the overactive oxygenation and oxidative stress in the mother and fetal immune interface of patients with RSA [32]. Overexpressed angiogenic growth factors cause aberrant endometrial angiogenesis and vascular disorder, including precocious development of endometrial blood vessels and lowered resistance of uterine artery to blood flow [33] and microvessel density, which above have been found to accumulate in women with RSA [34]. A recent study has shown that obesity is associated with adverse reproductive outcomes. This is because a high-fat diet, which is related to impaired vascular remodeling within the uterus, promotes uNK cell activation during pregnancy and altered uNK gene expression [35].

In recent years, research on autophagy has become popular in the immunological field. Autophagy, a firmly controlled catabolic approach of cellular self-degradation, is defined as a non-apoptotic form with relevance in overstimulated programmed cell death resulting from the stimuli-initiation [36], and it is substantially a cellular tension reaction and quality regulation mechanism [37]. Autophagy has an important effect on embryonic growth during the early stage of pregnancy. This development is usually associated with reproductive disorders, including abortion and preeclampsia [38, 39]. A recent study reported that the level of autophagy in the villi of RSA sufferers was remarkably lower than that of selective termination in pregnant women, and suggested that the suppression of trophoblast autophagy causes RSA via IGF-2 secretion and PEG10 reduction [40]. This study demonstrated that a high level of IGF-2 leads to NK cell transformation into a special category of cell with high cytotoxic activity, which then attacks normal cells at the immune interface. The latter has a negative influence on the process of vascular invasion, which induces pregnancy failure [40].

A previous study identified a new type of NK cell producing IL-22, named NK22, in the intestinal mucosa [41]. By 2010, NK22 cells were also found in the uterine mucosa [42]. In addition, Kamo et al. indicated that during endometrial mid-secretory stage, the percentage of NK22 cells was higher in a group of patients with RSA than in those suffering from unexplained infertility (UI) [43]. To date, the mechanism involved in these observations is not entirely clear. However, a study has shown that the proliferation of human trophoblastic cell line HTR8/SVneo is fueled by IL-22 [44]; this cytokine also inhibits apoptosis of trophoblastic cell. And NK22 cells

may play a major role in RSA at the maternal-fetal interface by producing IL-22, fueling proliferation, boosting cell variability, and inhibiting paracrine trophoblast apoptosis [45].

## Uterine Dendritic Cells

In the decidua, uterine DCs are believed to play a key role in the delicate equilibrium involved in maternal recognition of paternal antigens. It has been suggested that in the aspect of differentiation of endometrial stromal cell, DCs play a positive role positive tropism, in proliferation, and local angiogenesis [46]. They are considered major regulators of the immune response, augmenting T cell-mediated immunity, and stimulating regulatory T cell induction. It is suggested that decidual DCs may also play a crucial role in the etiology of RSA [47]. Any disturbance in their distribution, maturation state, or function might have a negative impact on pregnancy outcome, leading to adverse pregnancy outcomes [48]. In recent human studies, the following findings have been demonstrated: (1) compared with the control group, the levels of myeloid DCs (MDCs), and CD86<sup>+</sup> DCs in the RSA group were increased significantly, and CD200 expression on peripheral blood DCs was significantly lower in the RSA group [49]; (2) an elevated number of mature DCs and a decreased quantity of immature DCs may be associated with RSA [50]; (3) and compared with controls, ILT4<sup>+</sup> DCs in the peripheral blood and endometrium were decreased in women with RSA [51].

## Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) have emerged as a new immune regulator at the maternal-fetal interface. They participate in regulating other immune cells, especially on T cells, by suppressing their activities [52]. We have two categories based on phenotypes: MDSCs (MO-MDSCs) and granulocytic MDSCs (GR-MDSCs). Recent studies have shown that GR-MDSCs generally accumulate at the maternal-fetal immune interface, and that their immunomodulatory properties may be significant for fetal-maternal tolerance [53, 54]. In one study, researchers demonstrated that GR-MDSCs accumulated in the human placenta in healthy pregnancies, while they were remarkably diminished in patients suffering spontaneous abortion [55]. MDSCs are likely to function in RSA by regulating hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ). Previous research has also shown that HIF-1 $\alpha$  expression was lower in the missed abortion group than in the elective abortion group [56]. Furthermore, scientists have extensively investigated the relationship between HIF-1 $\alpha$ , MDSCs, and RSA, and recent research provides a reasonable explanation for the above phenomena. Myeloid cells with deficient HIF-1 $\alpha$  cause a diminishing accumulation of MDSCs, diminish the

suppressive activity of MDSCs, increase the apoptotic rates of MDSCs, and enhance the abortion rate [57]. It is also known that MDSCs are myeloid cells with suppressive activity on other immune cells. Therefore, the alteration of the number and function of MDSCs has a negative influence on fetal-maternal immune tolerance. Furthermore, research clearly suggests that in early miscarriage (EM) patients, the decline in G-MDSCs is related to a decline in estrogen (E2) and progesterone (P4) [58]. Women suffering from EM also generally experience poor endometrial receptivity, due to the downregulation of ER- $\alpha$  and contravariant expression of caspase-3 in endometrium decidua [59].

Neovascularization is crucial for decidualization and establishment of the placenta. Previous research has reported that MDSCs are associated with the progression of neovascularization [60]. They facilitate nonimmune reactions such as angiogenesis by secreting the key pro-angiogenesis inducer VEGF during pregnancy. Thus, we speculated that in RSA patients, the reduction of GR-MDSCs might lead to deoxygenation disorder and embryo loss. A study confirmed this speculation, demonstrating that higher early miscarriage incidence is associated with a decrease in suppressive monocyte levels in the peripheral blood and endometrium of pregnant mice and women [61, 62]. Immune cells always form an interactive network rather than being isolated in the immune system. For example, pregnancy loss as a possible result of myeloid-derived suppressor cell depletion is associated with the upregulation of decidual NK cell cytotoxicity [63]. Meanwhile, a remarkable feature of a successful pregnancy is the higher frequency of non-cytotoxic NK cells and lower number of cytotoxic NK cells present at the maternal-fetal interface [63]. Some studies show that MDSCs could not only suppress DC and T cell maturation but also supported uNK cells and resting macrophage development [61]. It has also been documented that MDSCs can induce Foxp3<sup>+</sup> T regulatory (Treg) cells by activating TGF- $\beta$  via the TGF- $\beta$ / $\beta$ -catenin signaling pathway [64]. The proportion of uNK cells and Treg cells was therefore significantly upregulated, revealing that as a new immunosuppressive network system, the MDSCs-NK-Treg axis plays a complex and crucial role in regulating fetal-maternal immune tolerance. MDSC-tolerogenic dendritic cells and Treg cells play a crucial role in supporting normal pregnancy and placenta formation, and may represent a new network system in maternal-fetal immunity [63]. Another study has shown that MDSCs not only suppressed T cells via reactive oxygen species (ROS) production but were also capable of inducing a shift toward Th2 cell subtypes in a cell-cell contact manner [65]. Moreover, these cells could reduce the presentation of L-selectin on immature T cells, inhibiting their trafficking toward lymph nodes and sustaining fetal-maternal tolerance [54]. Placental GR-MDSCs play a negative regulatory role in T cell responses by expressing arginase I and producing ROS [66]. In fact,

GR-MDSCs can be sensitized at the immune interface between mother and embryo through interaction with trophoblasts [67]. Moreover, GR-MDSCs isolated from placenta polarized CD4<sup>+</sup> T cells toward a Th2 cytokine response [68]. As mentioned above, due to the importance of MDSCs in pregnancy, their absence or dysregulation may cause complications.

## Macrophages

Macrophages are a type of immune cell crucial for human pregnancy, present significant plasticity in response to various environmental signals, and regulate a series of reproductive events including implantation, placentation, fetal development, parturition, and, in particular, vascular remodeling at the maternal-fetal interface [69]. In the early stages of gestation, macrophages tend to invade trophoblasts and the spiral arteries [46], thus promoting embryo implantation, trophoblast invasion, and vascular remodeling, in addition to removing apoptotic cells and cellular debris [70–73]. The polarity and activity of decidual macrophages contributes to the above-mentioned functions. Decidual macrophages have been differentiated into classically activated macrophages (M1) and alternatively activated macrophages (M2). The former are characterized by higher antigen presentation, dominant proinflammatory responses, and the promotion of inflammation and tissue damage. On the contrary, M2 cells are responsible for the anti-inflammatory profile, and fuel tissue remodeling and repair. Decidual macrophages lean toward M1 polarization before blastocyst peri-implantation [74]. After implantation, particularly when trophoblasts begin to invade the uterine myometrium, a mixed M1/M2 profile state would account for a major part of the cell population [75]. Until the end of placental development, decidual macrophages transform into a predominantly M2 phenotype in order to protect the fetus and placenta until parturition [76].

An appropriate proportion of M1/M2 has a vital impact on successful pregnancy. An imbalance in this proportion inevitably leads to reproductive complications including RSA, Preeclampsia (PE), and intrauterine growth restriction (IUGR) [70, 77]. There is evidence that M2 macrophages are present in high numbers in the endometrium during the luteal phase and normal pregnancy [70]. M2 polarization has a significant function in the early stage of a successful pregnancy, and a decrease in the M2 macrophage population is closely associated with RSA [70]. Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), which is expressed by maternal macrophages [78], is involved in cell proliferation, cell differentiation, and organogenesis [79]. A previous study has suggested that this receptor is closely associated with M2 polarization. A recent study [80] has also been reported that decidua basalis macrophages are practically PPAR $\gamma$ -negative, and the

reduction of PPAR $\gamma$  in decidual macrophages appears to have a poor impact on M2 polarization [81]. This then leads to functional defects in immune regulation and the disorder of homeostatic properties. PPAR $\gamma$  downregulation may be associated with a specific inflammatory response against the fetus, both of which finally cause recurrent miscarriage.

On the other hand, as aforementioned, M1 macrophages mediate inflammatory responses by producing copious amounts of proinflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$  [82]. Among these, TNF- $\alpha$  participates in the mechanism of RSA by regulating stathmin-1 (STMN1) expression [83]. STMN1 is a microtubule regulatory protein involved in trophoblast proliferation and invasion. The presentation of STMN1 is regulated by TNF- $\alpha$ , which is secreted by M1 macrophages in a negative feedback loop. In women with RSA, M1 macrophages are abundant in the deciduae of RSA (M1) [70]. An abnormally elevated level of TNF- $\alpha$  causes STMN1 downregulation, which may promote RSA and decrease trophoblast proliferation and invasion by regulating the E-cadherin/ $\beta$ -catenin pathway [84].

Recently, miRNAs have attracted much attention in the context of RSA development [85–87]. miRNAs have been associated with a succession of cellular biological behavior, including cell differentiation, proliferation, migration, invasion, and apoptosis [88, 89], and they contribute to RSA by inducing macrophage polarization to the M1 subtype. A recent study showed that miR-103 expression is significantly downregulated in patients with RSA, with an increased trend in the expression of signal transducer and activator of transcription 1 (STAT1), which is a key factor in M1 polarization [90]. The downregulated miR103 enhances M1 polarization by the STAT1/IRF1 pathway, which aggravates RSA. miR-103 may therefore be a diagnostic marker and promising therapeutic target for RSA [91]. and miR-103 may therefore be a diagnostic marker and promising therapeutic target for RSA.

Macrophages also induce Fas ligand (FasL)-mediated apoptosis, and it is known that apoptotic deregulation is associated with recurrent miscarriage (RM) [92]. The Fas/FasL system is a typical apoptotic pathway in cells and tissues [93]. Among the current researchers, CD68 (a marker for macrophages), CD163 (M2 marker), and CD86 (M1 marker) were used to characterize macrophage populations in the decidua; in the RSA patients, the population of CD68<sup>+</sup> macrophage in the decidual tissues of patients with RSA was remarkably increased, followed by an increased population of CD86<sup>+</sup> macrophage and a reduced population of CD163<sup>+</sup> macrophage, compared with the control subject [92]. A previous study suggested that FasL expression is significantly upregulated on the surface of CD68<sup>+</sup> and CD86<sup>+</sup> macrophages [94]. The binding of the Fas receptor by FasL activates a cascade of intracellular proteolytic enzymes, leading to Fas-bearing cell apoptosis. This may, therefore, be closely associated with the mechanism of RSA. In addition, at the maternal-fetal

interface, macrophages interacting with other immune cells constitute a huge network of cellular connections and participate in RSA development. For instance, it has been suggested that CD14<sup>+</sup> decidual macrophages enhance fetal tolerance by reducing the cytolysis of dNK cells and adjusting adaptive T cell responses [95].

A key study has demonstrated that an appropriate level of histone deacetylase 8 (HDAC8) has a positive effect on macrophage polarization and apoptosis [96]. This maintains successful pregnancy by elevating the presentation of M2 marker genes and inhibiting macrophage apoptosis at the maternal-fetal interface [97, 98]. The results of this study also suggested that HDAC8 mRNA and protein expression was downregulated in decidual macrophages in RSA patients [99]. This may lead to a decrease in M2 polarization and an increase in decidual macrophage apoptosis, which further contributes to RSA development.

## T cells

T cells have a significant role in the human immune system, and are present in most organs, particularly at the maternal-fetal interface. These cells play an irreplaceable role in maintaining normal pregnancy. When aberrations occur in the number or differentiation of T cells, this is closely associated with serious dysfunction of the reproductive system, including RSA [100]. A large amount of research on T cells has been performed over the last century. Researchers have divided T cells into three groups based on the different markers presented on T cell surfaces: CD4<sup>+</sup> T helper cells, CD8<sup>+</sup> T cytotoxic cells, and CD4<sup>+</sup> CD25<sup>+</sup> T regulatory cells [101]. These three categories of cells present different functions in the human immune system. CD8<sup>+</sup> T cytotoxic cells can directly cause cell death [102]. In the decidua, CD8<sup>+</sup> T cells can also produce effector cytokines and regulatory molecules. A significant elevation in the proportion of decidual CD8<sup>+</sup> cells was observed in RSA patients, which may also be related to the poor balance between maternal-fetal immune tolerance [103]. Human CD4<sup>+</sup> T helper cells assist in regulating the immune response, particularly in pregnancy and delivery [104], by releasing a large amount of cytokines.

Recently, with the further study of CD4<sup>+</sup> T helper cells, we know that functionally distinct Th cells, including Th1, Th2, and Th17 cells, will be induced when naive T cells are stimulated via T cell receptor engagement in conjunction with co-stimulatory molecules and cytokines produced by innate immune cells [105]. These usually have a distinctive impact on the human immune response. For instance, Th1 cells are responsible for cell-mediated immunity [106]. They are able to regulate the development of NK cells, lymphocyte activated killer (LAK) cells, cytotoxic T lymphocyte (CTL) cells, and kill trophoblasts [107]. Recently, much attention has been

paid to T cell immunoglobulin domain and mucin domain-3 (Tim-3) in the field of reproductive immunity. Tim-3 is expressed in differentiated Th1 cells, and a recent report showed that the intensity of Tim-3 expression was higher in RSA patients [108]. This is consistent with a previous study, which reported that Tim-3 regulated the Th1 cell-mediated immune response and is associated with autoimmune diseases [109]. Th2 cells participate in humoral immunity [110], and Th17 cells are associated with both the maintenance of mucosal barriers and autoimmune anomalies [111]. There is evidence that Th17 cell differentiation is related to the suppression of Th1 development by means of a considerable number of cytokines. This includes TGF- $\beta$  [112], which is usually associated with the pathogenesis of autoimmune diseases such as RSA [105]. One study demonstrated that minimal skew toward a Th2 population improves the outcome of normal pregnancy [106]. However, other researchers strongly believe that an extreme shift toward Th2 polarization leads to a higher abortion rate [113]. Thus, a precise balance may be involved in the polarization of Th1 and Th2 cells. Most scientists believe that maintaining an appropriate increase of Th2 is necessary for a successful pregnancy [107, 114]. Moreover, vitamin D may have an integral role in changes in the T cell subsets [115, 116]; the positive impact of vitamin D on shifting the cell population to Th2 predominance and enhancing fetal-maternal tolerance has been demonstrated [117]. Consistently, a recent study showed that the levels of vitamin D receptor in the villi and decidual cells of RSA patients, and even in the serum, were significantly lower than in control women [118].

In normal pregnancies, the reactions of both Th1 and Th2 cells against paternally derived antigens are suppressed by CD4<sup>+</sup> CD25<sup>+</sup> Treg cells [119]. Treg cells have important functions in self-tolerance, allograft tolerance, and the establishment of immune tolerance at the maternal-fetal interface [120, 121], and a decrease in the level of Treg cells causes unfavorable pregnancy outcomes [122]. The mechanisms by which Tregs contribute to RSA primarily involve an imbalance in the Th1/Th2/Th17/Treg cell paradigm, in addition to abnormal Treg cell proportion and activity [123]. In recent years, the role of Treg cells in the maternal-fetal interface has become a primary focus of research in the field of reproductive immunity [123–127]. The accumulation of Treg cells at the maternal-fetal interface during early gestation has been associated with the interactions between many cells. In the early pregnant uterus, for settling in the decidua, Treg cell expansion and movement are induced by human chorionic gonadotropin (hCG) [128]. Human chorionic gonadotropin also increases the restrictive capability of Treg cells [129]. Reproductive hormones in females are likely to play predominant roles in driving these dynamic Treg cell shifts [130]. Interestingly, a previous meta-analysis also indicated a statistically significant reduction in miscarriage rate using hCG

[131]. Treg cell alteration is not only associated with female reproductive hormones but also with the BMI of pregnant women. An interesting study reported a negative correlation between BMI and levels of activated Treg cells in the peripheral blood. [130]. Obesity is a growing public health problem associated with a significant increase in the risk of miscarriage [132]. In addition, Treg differentiation was also achieved by endovascular extravillous trophoblasts (enEVTs), which are capable of establishing placental-maternal circulation during human pregnancy [133]. However, a recent study observed that a significant decrease in the percentage of enEVTs was associated with the failure of Treg cell differentiation, leading to insufficient spiral artery remodeling in RSA patients [134].

Tregs are associated with the downregulation of an excessive inflammatory response for the implanting embryo and fetus [135]. Although Treg cell dysfunction has been confirmed to be associated with RSA, the key mechanisms involved have not been elucidated yet. [136]. Foxp3+ Tregs have been identified as a primary focus in the pathobiology of RSA. This is based on evidence that the average intensity of Forkhead box transcription factor P3 (FOXP3) and FOXP3 mRNA expression in Tregs were dramatically lower in patients with unexpected recurrent spontaneous miscarriage ( $P < 0.01$ ) [137]. Downregulated FOXP3 expression in Tregs was concomitant with autoimmune diseases [138]. Upregulated FOXP3 expression can facilitate the transformation of naïve T cells into Tregs [139], while FOXP3 downregulation was associated with immunorestrictive Treg dysfunction [140]. However, in a recent review, Li et al. instead focused on the FoxP3-negative Treg subtype and demonstrated its possible role in RSA [141]. Furthermore, STAT proteins play a conspicuous role in the epigenetic control of T cell differentiation [142]. For instance, it has been reported STAT3 had a crucial impact on the function of decidual Treg cells in RSA patients [143]. This stimulation state repressed Treg cell function, leading to a reduction in Treg cell maturation and restraint [136]. Moreover, STAT5 deficiency leads to loss of Foxp3+ Tregs [144]. The balance between Tregs and Th17 cell differentiation appears to be regulated by STAT5 and STAT3 [145]. In addition, researchers have found that impaired adaptive immune responses in patients with COVID-19, as a result of infection with SARS-CoV-2, lead to immune system dysregulation [146]. This involves increased levels of Th17 cells and a decrease in the number of Treg cells [127]. The immune imbalance of Th17 and Treg cells may therefore play an essential role in RSA [147].

A number of complex interactions and equilibrium among various cell types contribute to the establishment of an immunotolerant environment at the maternal-fetal interface [148]. For example, it has been suggested that ILC2s, which modulate T cell function by inducing Th2 cell activation, are correlated with Treg function and RSA [149]. Moreover, as mentioned previously, unbalanced immune responses and

deregulated function of T cell subtypes cause RSA [147], and this is usually associated with a discrepancy in T cell expression and the miRNA profile [150]. A significantly reduced level of T cells may be associated with insufficient decidualization of the endometrium, which is a crucial factor in the mechanism of RSA [151]. However, there is no clear and immediate relationship between RSA and other T cell subtypes, including peripheral blood CD3 T cells. A recent study reported that a nonlinear relationship has been observed between the proportion of CD3 T cells and risk of RSA [152]. If a successful pregnancy cannot achieve equilibrium between proinflammatory and anti-inflammatory immune responses from the maternal-fetal interface, this will inevitably lead to poor pregnancy outcomes [153].

## Treatment of Recurrent Spontaneous Abortion

Vast studies on unexplained RSA (URSA) suggested potential immunologic modification, containing underlying autoimmune inflammation [154]. Aspirin, low molecular weight heparin (LMWH), and an association of them have been applied in research for URSA as treatments. [155, 156]. There is an evident case of an autoimmune progress result in RSA that combination therapy with low-dose aspirin and prophylactic heparin or LMWH could bring better pregnancy outcomes in patients with antiphospholipid syndrome, which has been taken as a standard therapeutic method for female meeting standard for obstetric antiphospholipid syndrome [157]. Furthermore, an existing evidence suggested that immunological therapeutic method had a positive role in RSA [154]. A variety of immunotherapies have become available, covering intravenous immune globulin (IVIG), immunosuppressant, and granulocyte colony-stimulating factor [158, 159]. Besides, other therapies which can affect the body immune status include progesterone and vitamin D supplementation [160, 161]. Lymphocyte immunotherapy (LIT) and intravenous immunoglobulin (IVIG) are widely used in the treatment of URSA patients [162]. And studies have suggested that the application of LIT7 and IVIG could increase the pregnancy rate. In IVF-ET patients who suffer repeated implantation failure, LIT7, and IVIG can enhance embryo implantation via balancing the Th1/Th2 [163]. They can also promote the percentage of T regulatory cells (Tregs) [164], and restrain the activity of natural killer cell (NK cell) [165]. Moreover, Cyclosporine A confirmly had an immunosuppressing role that reduces the percentage of Th17 cells and promotes Treg-cell dominance through altering the expression of co-inhibitory molecules in patients with RSA [166]. Moreover, progesterone is critical for the maintenance of pregnancy. Previous researches have proposed that supplementation of progesterone may decrease the risk of

miscarriage in patients suffering recurrent or threatened abortion [167]. This may be related to the mechanism of progesterone. Progesterone plays a critical role in maintaining a successful pregnancy mediated by inducing secretory changes in the lining of the uterus, inhibiting the contraction of the uterine, enhancing the perfusion of the circulation in utero-placenta, and regulating mother immune responses [168]. The progesterone takes its physiologic effects by interacting with its receptors, progesterone receptors (PRs)[169], including nuclear progesterone receptors (NPRs) and membrane progesterone receptors (mPRs) [170, 171]. One supporting study proved that in the RSA patients, the expression of mPR- $\beta$  in the endometrium was remarkably lower than the normal control subject. During pregnancy, extravillous trophoblasts (EVTs) establish blood supplyment for the growing fetus by invading the maternal decidua with remodeling the local vasculature [172]. The expression of HSD3 $\beta$ 1, the key enzyme in the generation pathway of progesterone, was found to be declined in EVT of patients suffering RSA, which implies the metabolism of progesterone is impaired in EVT of female with miscarriages[160]. It was determined that progesterone had certain immunomodulatory effect via recovering the levels of different co-inhibitory molecules, containing T cell immunoglobulin mucin family member-3, cytotoxic T lymphocyte-associated protein-4, in the management of RSA [166]. Of cause, the present researches are limited with studies being small, and lots of further experiments are needed.

## Conclusions

Unexpected recurrent spontaneous miscarriage is a major and complex complication of female reproductive imperfection, and affects numerous women of childbearing age. Based on previous research, immunodeficiency accounts for an overwhelming portion of the multiple factors leading to unexpected recurrent spontaneous miscarriage. The multiple interactions among various immune cells may affect the balance between immune activation and embryonic antigen tolerance via many possible mechanisms, thus causing the development of RSA. In this review, we summarized and discussed existing research on immune cells related to RSA to enhance our understanding of this condition. Improving our knowledge on RSA will facilitate the development of new preventative or treatment measures.

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## Declarations

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