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CD154 Promotes Recurrent Spontaneous Abortion by Regulating Th17/Treg

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KEYWORDS CD154. IL-17-producing T Helper. Recurrent Spontaneous Abortion. Treg. Treatment

ABSTRACT The researchers aimed to find the mechanism of IL-17-producing T helper (Th17) or Treg imbalance in recurrent spontaneous abortion (RSA). Peripheral blood was collected from 8 RSA patients and 6 healthy pregnant women, and peripheral blood mononuclear cells were isolated. Flow cytometry was used to analyse the expression of Th17 cells, Treg cells and CD154 molecules in lymphocytes. Compared with healthy pregnant women, Th17 cells and CD154 in peripheral blood mononuclear cells of RSA patients were significantly increased, while Treg cells were significantly decreased. The up-regulation of CD154 can lead to Th17/Treg imbalance and RSA, and can provide a new direction for the treatment of RSA.

INTRODUCTION

Recurrent miscarriage (RSA) refers to the loss of a fetus before 28 weeks of pregnancy with the same sexual partner twice or more, and is a common pregnancy complication in obstetrics and gynecology. The etiology of RSA is complex, among which the disorder of the endocrine system is a risk factor leading to an increased risk of miscarriage, and insulin resistance (IR), as an important component of endocrine abnormalities, plays a particularly significant role. Any imbalance in maternal immune response leading to immune tolerance deficiency may result in the embryo being attacked by the maternal immune system, causing apoptosis of the decidual tissue invaded by trophoblast cells and inhibiting their invasive ability, thereby triggering miscarriage events (Atik et al. 2018). Current studies have found that maternal immune factors (including autoimmune and allogeneic immunity), thrombosis-prone factors (including hereditary and acquired thrombosis susceptibility), uterine anatomical abnormalities and endocrine abnormalities are important causes of RSA (Abdollahi et al. 2015). Among them, 80 percent of unexplained abortions are closely related to immune factors (Abdollahi et al. 2015).

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The main cells responsible for maternal fetal immune tolerance are decidual immune cells from the mother, natural killer cells (NK) from the uterus T cells and macrophages are abundant in the decidual tissue, playing a role in regulating epithelial embryonic attachment. The decidua tissue is a crucial component of placental and fetal development during early pregnancy and is closely related to RSA. Therefore, analyzing and screening the main genes and key pathways of decidua tissue in RSA combined with IR patients can help identify potential biomarkers and therapeutic targets. High throughput sequencing, with its advantages of speed, accuracy, low cost, wide coverage, and high output, has become the preferred method for biological process analysis and transcriptomics research. Due to the limited availability of tissue samples from RSA combined with IR patients, and the existing limitations in commonly used examination methods.

Th17 cells have the ability to induce inflammation and mainly secrete cytokines such as IL-17. Th17/Treg forms a group of "pro-inflammatory/anti-inflammatory" immune cell pairs, directly regulating the mechanism of immune response (Saifi et al. 2014). Therefore, understanding the role of Th17 and Treg in RSA is crucial for the treatment of RSA.

According to the cytokines secreted by them, regulatory T cells (Treg) and Th17 cells, which play different biological functions (Wang et al. 2020). Among them, Treg comprehensively maintains the stability of the immune system in mater-

nal-foetal immune tolerance (Liu et al. 2020). The number and function of Treg in normal pregnant women were increased. The decrease in the number of Treg and dysfunction are related to unexplained recurrent spontaneous abortion. Th17 cells are closely related to Treg cells, regulate and transform each other, organ transplant rejection and RSA (Wu et al. 2014). RSA is closely related to the balance of Th17/Treg.

CD154 is the ligand of CD40, CD154-CD40 pathway is one of the key pathways required for the complete activation of T cells during allogeneic immune response. Cell surface and soluble CD154 were mainly expressed by activated CD4+T cells (Cron 2003). CD154 blockade can effectively inhibit primary allogeneic active CD4+T cell response (Iwakoshi et al. 2000). In the process of allogeneic organ transplantation, the lack of CD154-CD40 costimulation can effectively prevent acute allograft rejection (Shimizu et al. 2000). Blocking CD154 can prevent atherosclerosis and atherosclerotic thrombosis (Hassan et al. 2009). It has been found that CD154-CD40 can regulate Treg and Th17 cells (Su et al. 2020a).

Objectives

The researchers aimed to find the mechanism of Th17/Treg imbalance in RSA.

METHODOLOGY

Patient Information

The study included RSA patients (n = 8) and control group (n = 6) from October 2022 to October 2023. Exclusion criteria for RSA patients were those with parental and embryonic chromosomal abnormalities and endocrine factors, female reproductive system without deformity or inflammation, prothrombotic state, no autoantibodies such as antiphospholipid antibody, antinuclear antibody, anti-DNA antibody, anti-sperm antibody and anti-thyroid antibody were found, and male semen is normal. Inclusion criteria were those with previous pregnancy loss that occurred with the same sex partner, there were two or more consecutive spontaneous abortions, and the gestational age was less than 10 weeks when they came to the hospital. The clinical information is shown in Table 1.

Table 1: Clinical information

Iteam	*NC n=6*	*RSA n=8*
Age	33.5±0.55	33.25±1.45
BMI	21.35±0.718	21.23±0.775
Number of abortions within 10 weeks	0.1667±0.4082	3

Flow Cytometry

Peripheral blood samples (10 mL) of all subjects were collected in the morning. The changes of Th17, Th1 and Treg cells were analysed. CD4-eFluor (5 μL), CD154-eFluor (5 μL), CD8-PE (5 μL) and CD25-APC-A (5μL) antibodies (Jiangsu Bitai Biotechnology Co. Ltd., China) were added to the test tube and incubated at 20 °C for 30 minutes. Cells were washed with PBS and resuspended in Fix or Perm buffer (eBioscience, USA), then incubated at 4 °C for 30 minutes in the dark. After washing with a Perm buffer twice, the cells were centrifuged and the supernatant was discarded. According to the manufacturer's instructions, IL-17-PE (5μL) and Foxp3-PE (5μL) antibodies were added and incubated at 4 °C in dark for 45 minutes for intracellular staining. Isotype controls were used for each antibody (eBioscience, USA).

Statistical Analysis

All results are expressed as mean ± standard deviation by GraphPad Prism 5 (GraphPad Software Inc., California, USA). The two groups were compared by independent t test. $P < 0.05$ indicates statistical significance.

RESULTS

Changes of CD4 + T and CD8 + T in RSA Patients

The researchers isolated PBMC from peripheral blood, and then isolated lymphocytes by flow sorting (Fig. 1A). In addition, there was no significant difference in the proportion of CD4 + T and CD8 + T in lymphocytes (Fig. 1B).

Th17 Cells Changed in RSA Patients

The expression level of IL-17 in CD4 + T cells in peripheral blood of RSA patients was significantly higher than that in the NC group (Fig. 2).

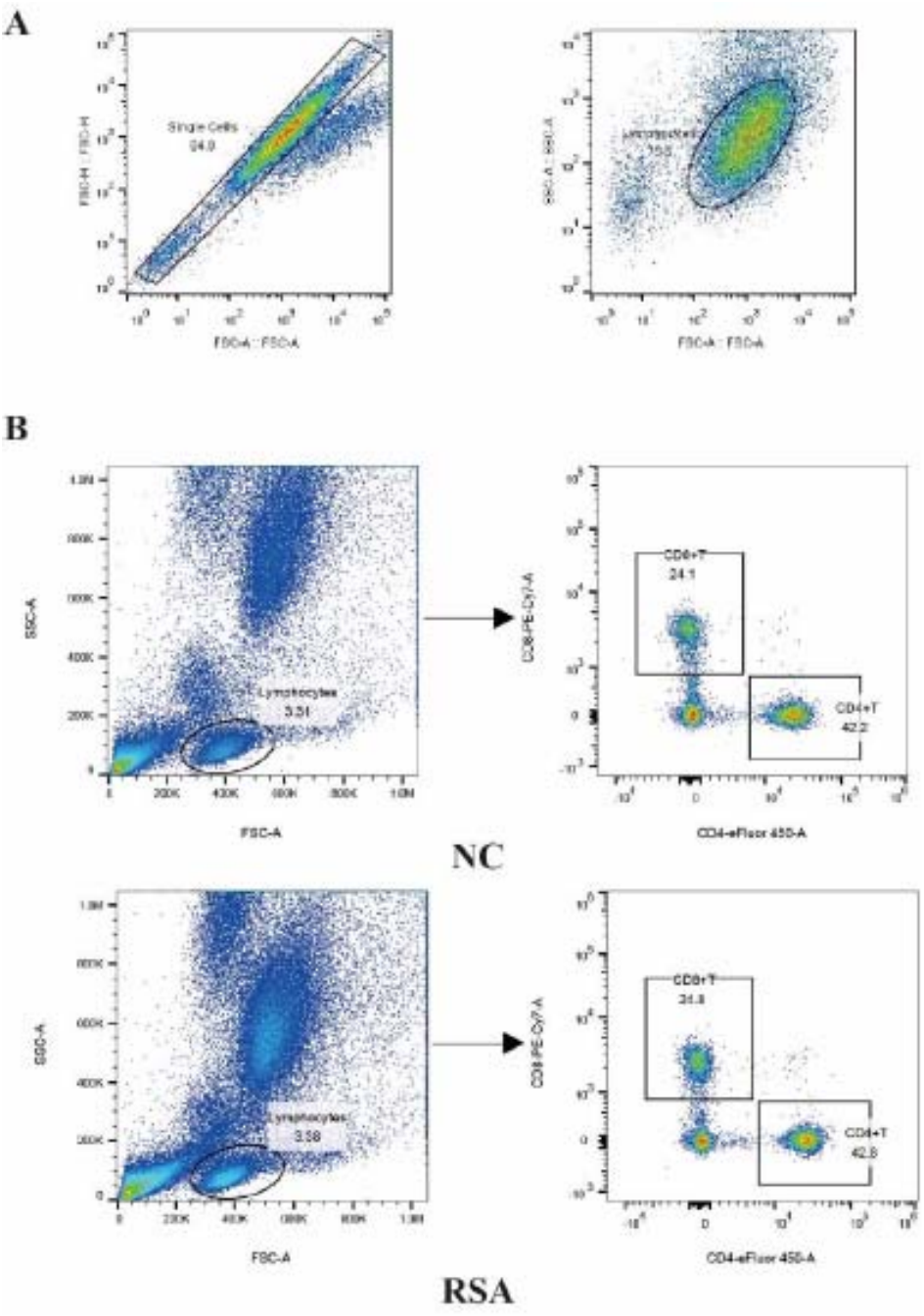

Fig. 1. The changes of CD4+T and CD8+T in RSA patients. A: Lymphocytes were isolated from PBMC; B: The proportion of CD4 + T and CD8 + T cells in lymphocytes

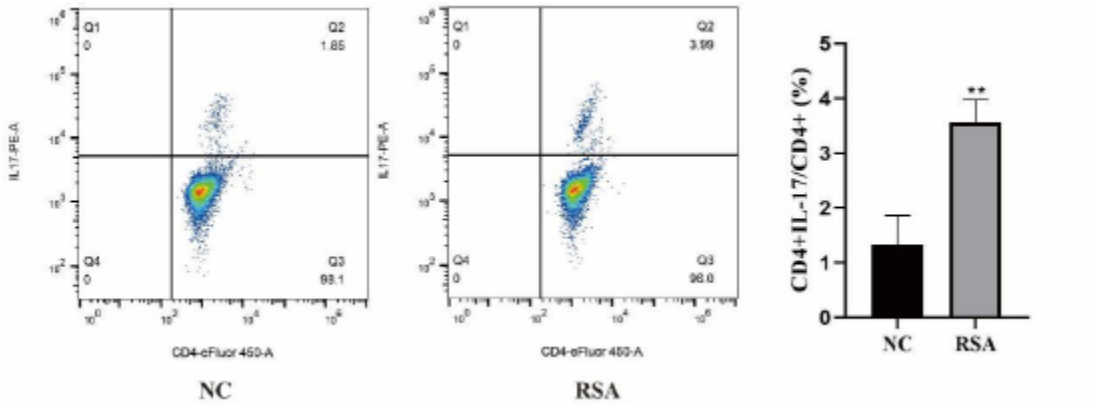

Fig. 2. The expression of IL-17 in peripheral blood CD4+T cells of RSA

CD154 Changed in RSA Patients

CD154 is mainly expressed in activated T cells. The results showed that CD154 was highly expressed in CD4 T cells in peripheral blood of RSA (Fig. 3).

Changes of Treg Cells in Peripheral Blood of RSA

Therefore, Foxp3 can be used as a marker to identify Treg cells. CD4+T cells were divided into three levels of CD25+high, CD25 low and CD25 negative. The researchers selected CD4+CD25 high cells for further detection of FoxP3+ expression levels. Compared with the NC group, the expression level of CD25+FoxP3 in CD4+T cells was significantly decreased in peripheral blood of RSA patients (Fig. 4).

DISCUSSION

Immune diseases are closely related to unexplained RSA (Dimitriadis et al. 2020). The maternal body shows physiological immune tolerance to embryonic semi-allogeneic antigens. Once the immune tolerance is unbalanced, it will lead to spontaneous abortion (Deshmukh and Way 2019). Revealing the exact mechanism of maternal-foetal immune tolerance and finding a new method to induce immune tolerance are the best ways to overcome immune rejection.

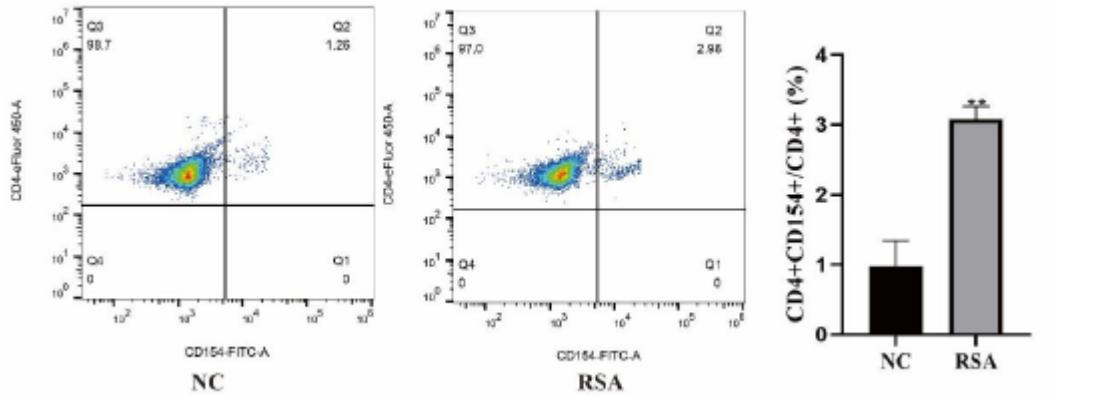

Fig. 3. The expression of CD154 in CD4+T cells

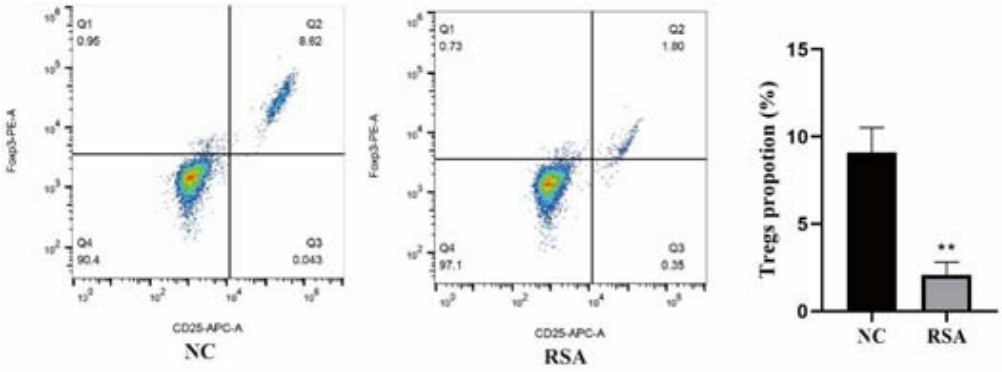

Fig. 4. The expression of FoxP3 in peripheral blood CD4+CD25 cells of RSA

At present, the incidence of RSA accounts for 1 percent to 5 percent of women of childbearing age, which can lead to anxiety, depression, increased economic burden, and seriously affect family and social stability. With the gradual decline of human fertility, the incidence and treatment of RSA have attracted much attention (Zhang et al. 2020). Treg cells maintain pregnancy through strong anti-inflammatory, immune regulation and vascular regulation (Tang and Hu 2023).

Resulting is in an imbalance of immune status between the mother and embryo, leading to RSA (Abdolmohammadi et al. 2019). The maternal fetal interface contains various immune related factors, mainly including the CD4+T cell family and its secreted cytokines. This can cause the maternal immune system to reject the embryo, affecting its development and ultimately resulting in miscarriage. The opposite is true for normal pregnant women (Huang et al. 2023). Studies have shown that the increase in the abundance of Th17 cells is not conducive to embryo implantation (Chen et al. 2021). The increase of Th17 cells can induce the activation of decidual cells and damage the blood vessels of uterine arteries, leading to embryo absorption (Travis et al. 2019). This study found that the expression of Th17 cells in peripheral blood of RSA patients was significantly increased, while Treg was significantly decreased.

CD40 is mainly expressed by B cells, dendritic cells, monocytes/macrophages and other immune cells (Díaz et al. 2021), while CD154 is only transiently up-regulated on activated T cells (Howard and Miller 2001; Ramsey et al. 2023). CD154-CD40 ligands play a central role in the development of immune response and T cell effector function, and are essential for maintaining the immune response (Díaz et al. 2021). The CD40-CD154 pathway is a co-stimulatory pathway that plays a role in the regulation of Th17/Treg balance (Xu and Song 2004). For example, cigarette smoke can promote the differentiation of mouse CD4 + T cells into Th17 cells through the CD40-CD154 co-stimulatory pathway (Liang et al. 2018). The upregulation of CD40 on dendritic cells is associated with Th17/Treg imbalance in young people with chronic periodontitis (Su et al. 2020b). In this study, the researchers found that CD154 was significantly up-regulated in CD4+T cells of RSA patients. Therefore, the researchers speculate that the increase of CD154 in RSA promotes the differentiation of Treg cells into Th17, resulting in the decrease of Treg cells and the increase of Th17 cells, thus promoting the occurrence of RSA.

CONCLUSION

CD154 is correlated with USRA. In RSA, the augmentation of CD154 facilitates the differentiation of Treg cells into Th17, leading to a reduction in Treg cells and an increase in Th17 cells, thereby promoting the occurrence of RSA. The findings of this study could offer a novel direction for the treatment of RSA.

RECOMMENDATIONS

CD154 might potentially be a novel therapeutic direction for RSA treatment, offering prospective targets for future clinical detection and treatment.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Cen Tang conducted research and wrote the article, and Wanqin Hu provided direction and ideas. Mingkun Shao and Xiaoyan Ma conducted the experiment. All authors have read and approved the final manuscript. Cen Tang is the First Author and Wanqin Hu is the Correspondent Author.

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