SHORT PAPER

Comparing Serum Levels of Th17 and Treg Cytokines in Women with Unexplained Recurrent Spontaneous Abortion and Fertile Women

Narges Roomandeh[1,2], AboTaleb Saremi[2], Javad Arasteh[3], Fatemeh Pak[4], Majid Mirmohammadkhani[5], Parviz Kokhaei[4,6]*, Ahad Zare[2*]

[1]Student Research Committee and Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, [2]Sarem Fertility and Infertility Research Center (SAFIR) and Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, [3]Department of Biology, Faculty of Basic Sciences, Islamic Azad University Central Tehran Branch, Tehran, [4]Cancer Research Center and Department of Immunology, Faculty of Medicine, [5]Research Center for Social Determinants of Health, Semnan University of Medical Sciences, Semnan, Iran, [6]Immune and Gene Therapy Lab, Cancer Centre Karolinska, Karolinska University Hospital, Stockholm, Sweden

ABSTRACT

Background: Increased evidences have shown that unexplained recurrent spontaneous abortion (URSA) is associated with inflammatory responses and breakage of immunological autotolerance. Therefore, the balance between Th17 and Treg cells may elucidate the pathophysiology of URSA. **Objective:** To investigate the serum concentration of regulatory and inflammatory cytokines associated with Treg and Th17 in both normal and URSA females. **Methods:** Forty-six women with URSA and 28 non-pregnant control women with at least one successful pregnancy were included. Serum was obtained from both groups and stored at -70°C. The serum concentrations of IL-17, IL-21, IL-22, IL-10, and TGF-β were quantitatively determined by ELISA. **Results:** The levels of IL-17, IL-21, and IL-22 in sera were significantly higher ($P<0.001$, $P=0.01$ and $P<0.001$, respectively) and TGF-β serum concentration was significantly lower ($P=0.02$) in URSA women compared with normal controls. **Conclusion:** Our results suggest that enhancement in Th17-associated cytokine levels and reduction in TGF-β may be one of the factors involved in URSA.

Roomandeh N, et al. Iran J Immunol. 2018; 15(1):59-67.

Keywords: Cytokines, Th17, Treg, Unexplained Recurrent Spontaneous Abortion

---

*Corresponding authors: Dr. Parviz Kokhaei, Cancer Research Center and Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, and Dr. Ahad Zare, Sarem Cell Research Center, Sarem Women's Hospital, Tehran, Iran, emails: p_kokha@yahoo.com and zare-a@alumnus.tums.ac.ir

INTRODUCTION

Owing to the fact that fetus expresses paternal histocompatibility antigens, it is considered as a semi-allograft that is not rejected in normal pregnancy (1). With the advent of reproductive immunology, a great deal of attention has been paid to the importance of maternal–fetal immune tolerance in normal pregnancy and its associated complications (2). Growing evidence shows that unexplained pregnancy loss is associated with inflammatory responses and breakage of immunological auto-tolerance (3,4). Unexplained recurrent spontaneous abortion (URSA) is a condition defined as the loss of three or more pregnancies prior to the 20th week of gestation with or without previous live births, occurring in 1–2% of human pregnancies (5,6). In most cases, the etiology of URSA is unknown; there exist, on the other hand, several established risk factors regarding such complication, including genetic and placental anomalies, endocrinological dysfunction, infection, uterine anatomical malformations, and hemostatic disorders (5,7). However, the etiology in approximately 50% of URSA is yet to be elucidated. Alloimmune and autoimmune mechanisms have been proposed as the immunologic factors in unexplained RSA (8). Depending on the cause, effective treatment is being performed on unknown pregnancy loss. Immune modulating therapies such as immunotherapy with paternal lymphocytes (9), intravenous immunoglobulin (IvIg) (10), intrauterine administration of autologous peripheral blood mononuclear cells (11), intralipids (12) and vitamin D are treatments that have been employed (13). Pregnancy loss is thought to be caused by the allorejection of fetus (14). It seems that Th1/Th2 imbalance with the predominance of Th1 immunity may also play a role in fetal loss (15). Th2-dominant immunity has been further observed in URSA cases (16,17). Accordingly, Th1/Th2 balance is not able to sufficiently explain the mechanism of the maternal immune response against fetus. IL-17 producing cells (known as Th17), which form a new subset of CD4+ T-cells, mediate the inflammation, autoimmunity, and immunological rejection of foreign tissues (18). These cells are closely related to Treg cells, which are important immune regulators controlling disastrous auto-reactive T-cells, particularly in the periphery (19). Several recent studies have suggested that a reduction in circulating Treg frequency is correlated with the maternal failure of fetus (20). Th17/Treg imbalance has also been observed in URSA patients (21). Moreover, it has been posited that pro-inflammatoryTh17 type cytokines promoting allograft rejection may compromise pregnancy, while the regulatory T-cell-related cytokines that promote fetal allograft tolerance may ameliorate the outcome of pregnancy (22,23). Cytokine balance during pregnancy conduces to the control of immune responses against fetal antigens for the survival of fetus. Based on the recent studies, the activation of STAT3 as a negative regulator of Treg cell differentiation, and the downmodulation of Smad2/3, Treg cell inducers, is induced by IL-21 (24). Moreover, a combination of IL-1, IL-6, IL-23, TGF-β, and IL-21 signaling is required for an optimal IL-17 production. Such findings corroborate the IL-21 contribution in human Th17 cell differentiation (25). Evidence has indicated the alteration of Th1/Th2/Th17 and Treg cells in unexplained recurrent miscarriage (3). In addition, changes in the pattern of cytokines produced by these cells play an important role in their balance.

In the present study, Th17 and Treg-related cytokine levels of women with URSA were compared with that of healthy women, aiming to improve the understanding of the pathogenesis of unexplained abortion. We determined the serum concentration of IL-17,

IL-21, IL-22, IL-10, and TGF-β in the two groups. Further evaluated in the research were IL-21 and IL-22 as a Th17 cytokines, where more samples were made use of comparisons to other studies on URSA women.

MATERIALS AND METHODS

Subjects. In this case control study, we included 46 women with a history of three or more sequential early abortions with mean age of 30.87 ± 5.39 years who referred to Sarem Women's Hospital, Tehran, Iran, between July 10, 2014 and December 1, 2014. Excluded from the study were patients with chromosomal abnormality, genetic disorders, infection (HBV, HSV, HCV, EBV, HIV and TORCH syndrome), autoimmune disease (presence of anti-cardiolipin antibodies, anti-nuclear antibodies, and anti-phospholipid antibodies), anatomical anomalies, uterine malformations, cervical incompetence, endocrinal abnormalities, and diabetes. As for the control group, we enrolled 28 non-pregnant healthy women with at least one successful pregnancy and no previous disease (spontaneous abortion, ectopic or abnormal pregnancy, preterm and post-term labor, preeclampsia, and endometriosis) and a mean age of 33.71 ± 5.054 years. The study was approved by the ethics committee of Semnan University of Medical Sciences and Sarem Women's Hospital and informed consent was obtained from all subjects prior to the enrollment.

Serum Preparation. Blood samples were taken from case and control groups at the implantation window during the luteal phase (at days 19–23 of the menstrual cycle) and centrifuged at 700 ×g for 10 minutes. The sera were aliquoted and stored at -70°C until cytokine analysis.

Detection of Cytokine Levels. The levels of IL-17A, IL-21, IL-22, IL-10 and TGFβ in serum samples were measured by enzyme-linked immunosorbent assay using ELISA kits following the manufacturer's instruction (eBiosciences, USA). The sensitivity of each assay was as follows: 0.01 pg/ml (IL-17A), 2 pg/ml (IL-10), 8 pg/ml (IL-21, IL-22), and 0.156 ng⁄ml for TGF-β. All the assays were run in duplicate.

Statistical Analysis. Statistical analysis was performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). Values are expressed as median and IQR (Inter quartile range). The data were evaluated by non-parametric Mann-Whitney U test in order to compare the cytokine levels between cases and controls. Receiver operator curve (ROC) analysis was employed to specify the cut off scores for positive IL-17, IL-21, IL-22, and negative TGF-β and IL-10 levels in RSA patients. To determine these cytokine scores, the sensitivity and specificity of each cytokine was plotted in RSA and control groups.

RESULTS AND DISCUSSION

Serum levels of IL-17a, IL-21, and IL-22 (as Th17-related cytokines) and Treg associated cytokines (TGF-β and IL-10) were detected in all the participants. IL-17 level was significantly lower in the serum of normal non-pregnant women, compared with URSA patients. As shown in Table 1, the concentrations of IL-21 and IL-22 were significantly higher in the sera of URSA patients. The difference in serum IL-10 levels in the two groups was not significant, but TGF-β as a Treg cytokine was significantly higher in the control group (Table 1).

Table 1. Serum concentrations of Th17 and Treg cytokines between URSA and control group.

Parameter	Mean difference		P value
	Control n=28	URSA n=46	
IL-17 (pg/ml)	0.40 (0 , 0.9)	1.9 (1.27,7.77)	<0.001*
IL-21 (pg/ml)	2.79 (0 , 13.5)	10.80 (5.5, 21.80)	0.017*
IL-22 (pg/ml)	3.01 (0 , 5.64)	17.45 (5.1 , 23.75)	<0.001*
IL-10 (pg/ml)	4.85 (3.87 , 6.01)	4.2 (2.4 , 9.32)	0.490
TGF-β (ng/ml)	26.41 (20.24 , 42.3)	14.22 (8.9 , 21.11)	0.021*

The values are expressed as Median (Inter quartile range). URSA: Unexplained Recurrent Spontaneous Abortion. P-value from nonparametric Mann-Whitney U test. *Significant difference (P<0.05).

Additionally, the ratio of IL-17, IL-21, and IL-22/TGF-β was analyzed in the total population of patients and controls, where the ratios of Th17/Treg cytokines in patients with URSA were significantly higher (Table 2).

Table 2. Th17/TGF-β cytokine ratios in patients with URSA compared with controls.

Parameter	Control n=28	URSA n=46	P value
IL-17/TGF-β	0.014 (0.0, 0.034)	0.21 (0.071 , 0.37)	<0.001*
IL-21/TGF-β	0.087 (0.0, 0.52)	0.56 (0.18,1.18)	0.003*
IL-22/TGF-β	0.086 (0.0, 0.24)	0.66 (0.27, 1.51)	<0.001*

The values are expressed as Median (Inter quartile range). URSA: Unexplained Recurrent Spontaneous Abortion. P-value from nonparametric Mann-Whitney U test. *Significant difference (P<0.05).

Receiver operator curves were generated to determine the cut-off values for optimal sensitivity and specificity of IL-17, IL-21 and IL-22 levels in RSA. They all proved to be fairly good predictors of URSA in our patient population (Table 3).

Table 3. Area under the ROC curve for cytokine measurements and the outcome.

Variable(s)	AUC	Std.Error	95% CI	P value
IL17	0.904	0.042	0.821-0.987	0.000*
IL21	0.666	0.066	0.536-0.795	0.017*
IL22	0.770	0.056	0.659-0.881	0.000*
IL10	0.453	0.068	0.320-0.585	0.497
TGF.β	0.339	0.063	0.216-0.463	0.021*

P-value from nonparametric Method. *Significant difference (P<0.05).

Patients with cytokine levels higher than these cut-off levels had a higher probability of developing URSA. The area under the curve (AUC) of IL-21, IL-22, and IL-17 was

<0.90. The area under the ROC curve (AUC) was further calculated to analyze the values of IL-17, IL-21, IL-22, IL-10, TGF-β levels and the IL-17, IL-21, IL-22/TGF-β ratio for URSA (Table 5). As shown in Fig. 1, ROC curve analysis demonstrates a higher AUC for the IL-17 of 0.904, IL-21 of 0.666 and IL-22 of 0.770 (P<0.017), compared with the IL-10 of 0.453 and TGF-β of 0.339 (P<0.49) (Table 3). Also, ROC curve analysis specified the fact that IL-17 level, with a sensitivity of 95.7% and a specificity of 82.1%, was the most useful cut-off point for the predictive diagnosis of URSA (Table 4).

Table 4. Area under the ROC curve for individual cytokine measurements and the presence of URSA.

Variables	Cut-off (pg/ml)	Fisher test P-value	OR (Odd Ratio)	95% CI	Sensitivity On Cut-off	Specificity On Cut-off
IL-17	1.05	0.000*	101.2	18.201-562.684	95.7%	82.1%
IL-21	5.0	0.000*	6.480	2.283-18.393	78.3%	64.3%
IL-22	6.65	0.000*	13.033	4.046-41.985	73.9%	82.1%

P-value from Fisher's exact test. *Significant difference (P<0.05).

Furthermore, the cut-off values of the optimal sensitivity and specificity for the IL-21 and IL-22 levels were determined by ROC regarding outcome (have successful pregnancy). These variables are all fairly good predictors of outcome in our patient population (Fig. 1, Table 4).

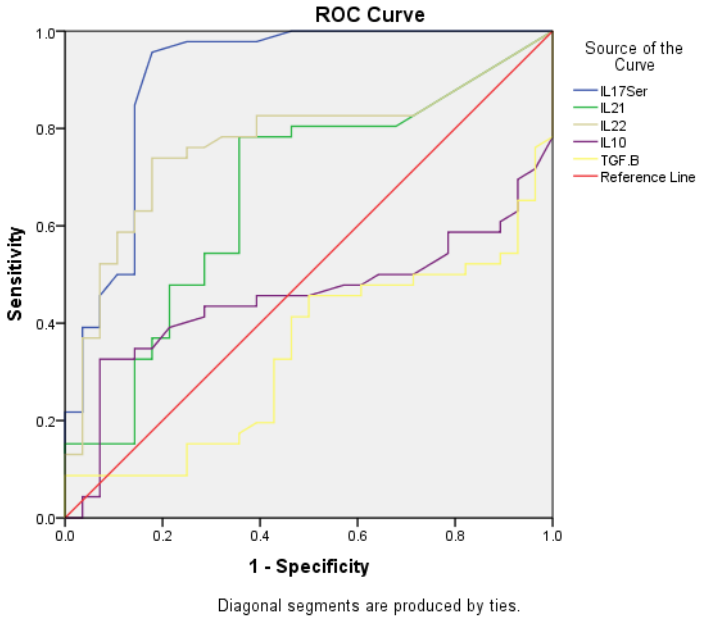

Figure 1. ROC curve analysis for the sensitivity and specificity of Th17 and Treg cytokines to predict URSA.

The AUC of IL-10 and TGF-β 1 was less that 0.45, hence the fact that these pro inflammatory mediators failed to predict URSA in our population (Table 3). The AUC of Th17 cytokines/TGF-β ratio was also identified (Fig. 2, Table 5).

Table 5. ROC curve analysis for the serum Th17 associated cytokines/TGF-β ratio.

Variable(s)	AUC	Std. Error	95% CI	P value
IL17/TGF	0.921	0.030	0.861-0.980	0.000
IL21/TGF	0.702	0.061	0.583-0.821	0.004
IL22/TGF	0.800	0.052	0.698-0.902	0.000

P-value from nonparametric Method. *Significant difference (P<0.05).

The cut-off point of the serum IL-17/TGF-β ratio was determined 0.043 pg/ml, revealing a sensitivity of 84.8%, and a specificity of 89.3 in predicting URSA (Table 6). The cytokines secreted by maternal cells are involved in the tolerance against the semi-allogenic fetus during pregnancy and may prevent normal implantation (6).

Table 6. Area under the ROC curve for Th17/Treg cytokines and the presence of URSA

Variable(s)	Cut-off (pg/ml)	Fisher test P-value	OR	95% CI	Sensitivity On Cut-off	Specificity On Cut-off
IL17/TGF	0.043	0.000*	46.429	10.970-196.493	84.8%	89.3%
IL21/TGF	0.194	0.001*	5.727	2.049-16.009	76.1%	64.3%
IL22/TGF	0.284	0.000*	14.636	4.494-47.670	76.1%	82.1%

P-value from Fisher's exact test. *Significant difference (P<0.05).

The function of Th17 as an effector cell is regulated by Treg cells. The balance between Th17 and Treg cells is necessary to preserve the implantation, hence a successful pregnancy (26). Increased levels of Th17 cells versus a decreased percentage of Treg cells indicates an immunological imbalance, which leads to pregnancy failure (6). In this study, we demonstrated that, compared with the controls, the serum levels of Th17-related cytokines, including IL-17A, IL-21, and IL-22 in patients with URSA were recognizably higher, while the concentration of Treg-associated cytokine (TGF-β) was significantly lower. Our results are in accordance with the previous studies that have indicated the protective role of Treg cells in the maintenance of pregnancy (23,26,27). In this regard, Lee *et al.* demonstrated that the level of IL-17-positive T-cells and ratios of Th17/Treg cells were significantly higher in the peripheral blood of non-pregnant women with idiopathic pregnancy loss than fertile controls (26). Recent studies have reported that the proportion of Th17cells in the peripheral blood and decidua is lower in pregnant women with URSA compared with parous controls (3,23). In addition, Wang

et al. showed that Th17 cells were enriched in peripheral blood and decidua in URSA patients and there was an inverse relationship between Th17 cells and Treg cells (3).

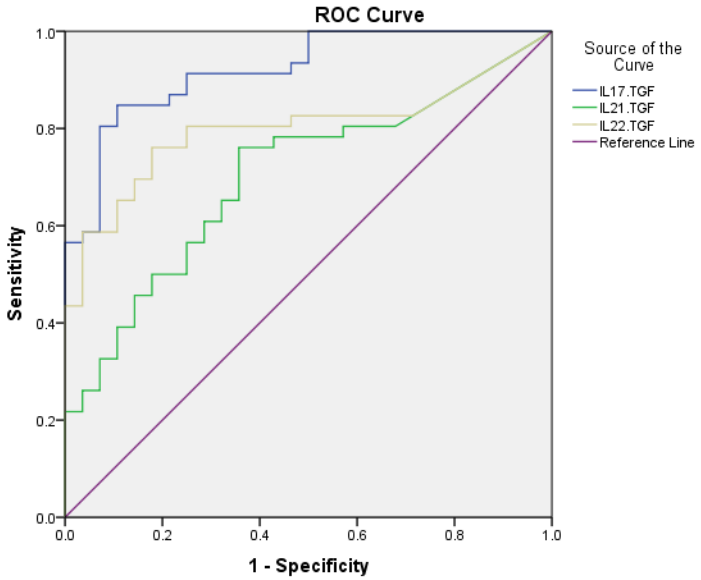

Figure 2. Area under the ROC curve, sensitivity and specificity of IL-17/TGF-β, IL-21/TGF-β and IL-22/TGF-β cytokines.

Therefore, Th17 cell-mediated inflammatory immune response is associated with URSA and Th17 cells may reverse the mechanisms mediating maternal immune tolerance of conceptus antigens, thereby resulting in the loss of pregnancy. In this regard, Saifi *et al.* reported that, compared with the normal non-pregnant group, the expression of IL-21 was higher in the URSA group while the expression of TGF-β and FoxP3 was lower (28). Such findings were further confirmed, in the present research, through the evaluation of Th17- and Treg-related cytokine concentration, where the levels of IL-17 and IL-21 were proved to be lower in URSA group. Several reports have indicated that the down-regulation of Treg cells leads to the failure in the immunological protection of the fetus and URSA patients show decreased levels of Treg cells both in the peripheral blood and in the decidua (29). Other investigations have suggested that TGF-β and IL-10 may act as mediators of suppressive function (30,31). Consistent with these findings, Wang *et al.* found that Treg cells can reduce IL-17 production, yet the suppressive ability was decreased in URSA. Furthermore, TGF-β and IL-10 were able to inhibit the expression of IL-17 and maintain a maternal-fetal tolerance during pregnancy (14), which is in line with the present study where normal women had a higher level of TGF-β. In another study by Wang *et al.* the rate of abortion, accompanied with a decrease in TGF-β and IL-10 production in mice, was increased after the administration of recombinant IL-17 and abnormal elevation of IL-17 expression in the feto-maternal interface, leading to abortion. The transfer of Tregs can also be effective in the prevention of URSA (32). Further adoptive transfer of IL-10-producing Tregs from pregnant mice increased IL-10 levels and decreased abortion rates in these mice (32). In

the present study, however, IL-10 levels were similar in both groups, which is in accordance with a recent study published by Yue *et al*. Consistent with the results of previous studies (23,28,33), we also found that the ratio of Th17/Treg cytokines was significantly higher in the URSA group .These results implicate cytokine shift from pro-inflammatory state to Treg cells in normal pregnancy, and highlight the importance of Th17/Treg balance, and their cytokine profile in pregnancy outcome.

In conclusion, we demonstrated that in patients with URSA, there is a significantly higher IL-21, IL-22, and IL-17 serum levels. Also, TGF-β (as a cytokine secreted by Treg cells) has a lower concentration in URSA patients compared with normal controls. Based on the sensitivity and specificity of these variables, our study underscores the importance of IL-17, IL-22, IL-21 and IL-17/TGF-β, IL-22/TGF-β and IL-21/TGF-β as useful indicators of URSA. It is possible that a slight decrease in Treg-related cytokines and an increase in Th17-associated cytokines, contribute to the pregnancy outcome of patients with URSA.

ACKNOWLEDGEMENTS

This work was supported by Semnan University of Medical Sciences, Semnan, under grant number 612 and Sarem Women's Hospital, Tehran, Iran.

REFERENCES

1. Erlebacher A. Why isn't the fetus rejected? Curr Opin Immunol. 2001; 13:590-3.
2. Li X, Wang B, Li Y, Wang L, Zhao X, Zhou X, et al. The Th1/Th2/Th17/Treg paradigm induced by stachydrine hydrochloride reduces uterine bleeding in RU486-induced abortion mice. J Ethno pharmacol. 2013; 145:241-53.
3. Wang WJ, Hao CF, Yi L, Yin GJ, Bao SH, Qiu LH, et al. Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. J Reprod Immunol. 2010; 84:164-70.
4. Bao SH, Wang XP, De Lin Q, Wang WJ, Yin GJ, Qiu LH. Decidual CD4+CD25+CD127dim/- regulatory T cells in patients with unexplained recurrent spontaneous miscarriage. Eur J Obstet Gynecol Reprod Biol. 2011; 155:94-8.
5. Carrington B, Sacks G, Regan L. Recurrent miscarriage: pathophysiology and outcome. Curr Opin Obstet Gynecol. 2005; 17:591-7.
6. Saini V, Arora S, Yadav A, Bhattacharjee J. Cytokines in recurrent pregnancy loss. Clin Chim Acta. 2011; 412:702-8.
7. Pandey MK, Rani R, Agrawal S. An update in recurrent spontaneous abortion. Arch Gynecol Obstet. 2005; 272:95-108.
8. Clark DA. Popular myths in reproductive immunology. J Reprod Immunol. 2014; 104-105:54-62.
9. Liu Z, Xu H, Kang X, Wang T, He L, Zhao A. Allogenic Lymphocyte Immunotherapy for Unexplained Recurrent Spontaneous Abortion: A Meta-Analysis. Am J Reprod Immunol. 2016; 76:443-453.
10. Wang SW, Zhong SY, Lou LJ, Hu ZF, Sun HY, Zhu HY. The effect of intravenous immunoglobulin passive immunotherapy on unexplained recurrent spontaneous abortion: a meta-analysis. Reprod Biomed Online. 2016; 33:720-736.
11. Yu N, Zhang B, Xu M, Wang S, Liu R, Wu J, et al. Intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) activated by HCG improves the implantation and pregnancy rates in patients with repeated implantation failure: a prospective randomized study. Am J Reprod Immunol. 2016; 76:212-6.

12. Dakhly DM, Bayoumi YA, Sharkawy M, Gad Allah SH, Hassan MA, Gouda HM, et al. Intralipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells. Int J Gynaecol Obstet. 2016; 135:324-327.
13. Bubanovic I. 1alpha,25-dihydroxy-vitamin-D3 as new immunotherapy in treatment of recurrent spontaneous abortion. Med Hypotheses. 2004; 63:250-3.
14. Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH, Lin QD. The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained early recurrent miscarriage. Hum Reprod. 2010; 25:2591-6.
15. Raghupathy R. Pregnancy: success and failure within the Th1/Th2/Th3 paradigm. Semin Immunol. 2001; 13:219-27.
16. Chaouat G, Ledee-Bataille N, Zourbas S, Ostojic S, Dubanchet S, Martal J, et al. Cytokines, implantation and early abortion: re-examining the Th1/Th2 paradigm leads to question the single pathway, single therapy concept. Am J Reprod Immunol. 2003; 50:177-86.
17. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010; 63:601-10.
18. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity. 2008; 28:445-53.
19. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nat Immunol. 2010; 11:7-13.
20. Winger EE, Reed JL. Low circulating CD4(+) CD25(+) Foxp3(+) T regulatory cell levels predict miscarriage risk in newly pregnant women with a history of failure. Am J Reprod Immunol. 2011; 66:320-8.
21. Sereshki N, Gharagozloo M, Ostadi V, Ghahiri A, Roghaei MA, Mehrabian F, et al. Variations in T-helper 17 and Regulatory T Cells during The Menstrual Cycle in Peripheral Blood of Women with Recurrent Spontaneous Abortion. Int J Fertil Steril. 2014; 8:59-66.
22. Piccinni MP. T cell tolerance towards the fetal allograft. J Reprod Immunol. 2010; 85:71-5.
23. Liu YS, Wu L, Tong XH, Wu LM, He GP, Zhou GX, et al. Study on the relationship between Th17 cells and unexplained recurrent spontaneous abortion. Am J Reprod Immunol. 2011; 65:503-11.
24. Battaglia A, Buzzonetti A, Baranello C, Fanelli M, Fossati M, Catzola V, et al. Interleukin-21 (IL-21) synergizes with IL-2 to enhance T-cell receptor-induced human T-cell proliferation and counteracts IL-2/transforming growth factor-beta-induced regulatory T-cell development. Immunology. 2013; 139:109-20.
25. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol. 2007; 8:967-74.
26. Lee SK, Kim JY, Hur SE, Kim CJ, Na BJ, Lee M, et al. An imbalance in interleukin-17-producing T and Foxp3(+) regulatory T cells in women with idiopathic recurrent pregnancy loss. Hum Reprod. 2011; 26:2964-71.
27. Yang H, Qiu L, Di W, Zhao A, Chen G, Hu K, et al. Proportional change of CD4+CD25+ regulatory T cells after lymphocyte therapy in unexplained recurrent spontaneous abortion patients. Fertil Steril. 2009; 92:301-5.
28. Saifi B, Rezaee SA, Tajik N, Ahmadpour ME, Ashrafi M, Vakili R, et al. Th17 cells and related cytokines in unexplained recurrent spontaneous miscarriage at the implantation window. Reprod Biomed Online. 2014; 29:481-9.
29. Jin LP, Chen QY, Zhang T, Guo PF, Li DJ. The CD4+CD25 bright regulatory T cells and CTLA-4 expression in peripheral and decidual lymphocytes are down-regulated in human miscarriage. Clin Immunol. 2009; 133:402-10.
30. La Rocca C, Carbone F, Longobardi S, Matarese G. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett. 2014; 162:41-8.
31. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science. 2008; 322:1562-5.
32. Wang WJ, Liu FJ, Xin L, Hao CF, Bao HC, Qu QL, et al. Adoptive transfer of pregnancy-induced CD4+CD25+ regulatory T cells reverses the increase in abortion rate caused by interleukin 17 in the CBA/JxBALB/c mouse model. Hum Reprod. 2014; 29:946-52.
33. Lee SK, Kim JY, Lee M, Gilman-Sachs A, Kwak-Kim J. Th17 and regulatory T cells in women with recurrent pregnancy loss. Am J Reprod Immunol. 2012; 67:311-8.