

Original Research Article

Effect of prednisone on IL-17 secretion in maternal-fetal immune rejection cell model, and on IL-23/IL-17 inflammation axis

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Abstract

Purpose: To investigate the influence of prednisone on secretion of IL-17 in maternal-fetal immune rejection cell model, and on tIL-23/IL-17 inflammation axis.

Methods: Four groups of Kunming mice were used. Group A was given blank culture solution. Group B was first given prednisone; thereafter, TGF- β , IL-23, and IL-6 were added. Groups C and D were first treated with culture solution containing TGF- β , IL-23, and IL-6. Then, prednisone was added to group C, while blank culture solution was added to group D. The mRNA expressions of IL-23 and IL-17 in mouse spleen lymphocytes were assayed in the four groups using their respective culture supernatants.

Results: The IL-17 concentration was significantly downregulated in group B, relative to the other groups ($p < 0.05$). Expression of IL-17 mRNA in mouse spleen lymphocytes was significantly downregulated in group B, when compared to the other groups, and was also higher in groups A and D than in group C ($p < 0.05$). After cytokine stimulation, IL-17 and IL-23 were upregulated in groups C and D. The expression level of mRNA was higher in D than in C ($p < 0.05$).

Conclusion: Prednisone inhibits the proliferation of lymphocytes. During immune imbalance, prednisone regulates immunity by controlling the secretion of IL-17 via downregulation of the expressions of genes for pro-inflammatory factors IL-17 and IL-23.

Keywords: Prednisone, Maternal-fetal immune, Pro-inflammatory factors, Lymphocytes

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INTRODUCTION

Abortion is a common complication of pregnancy. Recurrent abortion refers a situation where two or more pregnancies are lost in the first 20 weeks of gestation due to complications. About 50 % of miscarriages cannot be unexplained. These are referred to as unexplained recurrent spontaneous abortions (URSA). Recurrent

implantation failure (RIF) and URSA are serious issues of concern for reproductive clinicians [1,2]. With deepening of relevant research, many scholars believe that immunological abnormalities are important causes of URSA. It is thought that maternal-fetal immune imbalance is crucial in the occurrence of URSA. Maternal-fetal interface is a cytokine network formed by various cytokines secreted by immune cells. The

cytokine network maintains the maternal-fetal immune balance. If the maternal fetal immune tolerance is impaired, pregnancy failure results [3]. Studies have shown that interleukins (ILs) 17 and 23 in peripheral blood and decidual tissue of URSA patients are significantly increased, relative to normal pregnant women. Moreover, the expressions of IL-17-related factors are greatly increased in patients with threatened abortion and gestational diabetes mellitus. As an effector cytokine of Th17, IL-17 induces and stimulates immune cells to secrete inflammatory cells, and promotes the accumulation of neutrophils for induction of inflammation. Being a factor required for the amplification and maintenance of Th17 cells, IL-23 also promotes pro-inflammatory effects by stimulating CD5+T cells to secrete IL-17 [4]. In the field of reproductive medicine, prednisone produces some degree of effectiveness in the treatment of URSA and RIF, but it has not been widely recognized [5]. Therefore, in the present study, *in vitro* cell culture was used to establish maternal-fetal immune rejection animal cell model which was used to investigate the effect of prednisone on the secretion of IL-17. The effect of prednisone on the IL-23/IL-17 inflammation axis, and the mechanism involved, were also investigated.

EXPERIMENTAL

Animals and reagents

Female Kunming mice (6 - 8 weeks old, weighing 20 - 25 g) were purchased from the Animal Experimental Center of Guangxi Medical University. Phosphate buffered saline (PBS) was product of Wuhan Dr Bioengineering Co. Ltd. Fetal bovine serum and RBM1640 medium were bought from Gibico, USA. Thiazole blue and DMSO were purchased from Beijing Solebao. Anti-CD3 and anti-CD28 were produced by eBioscience, US; murine TGF- β factor, murine IL-23 factor, and murine IL-6 factor were products of Peptotech, USA.

Prednisone was a product of Zhejiang Xianju Pharmaceutical Co. Ltd, while IL-17a ELISA kit was produced by Lianke Biotechnology. The other equipment and their suppliers (in brackets) were: carbon dioxide incubator (Japan Sanyo); fully automatic microplate reader (BIO-Red, USA); sterile 96-well plate and 25-ml cell culture flask (Corning, USA); cryogenic high-speed centrifuge and pipettes of various ranges (Eppendorf, Germany); micro-ultraviolet spectrophotometer (Nanodrop 2000 Thermo, USA), and PCR (Polymerase Chain Reaction) instrument (Stratagene, USA).

This research was approved by the Animal Ethical Committee of Renmin Hospital (approval no. 2018812), and carried out in line with the "Principles of Laboratory Animal Care" (NIH, 1985) [6].

Kunming mice were sacrificed via cervical dislocation, and the spleen lymphocytes were isolated and used for preparation of single cell suspension which was stimulated to proliferate *in vitro* using anti-CD3 and anti-CD28 antibodies. The effect of prednisone at various concentrations (0.001, 0.01, 0.1, 1, 10, 100 and 1000) on the proliferation of the spleen lymphocytes was determined with MTT assay.

Kunming mice were divided into four groups (A – D). Group A was given blank culture medium. Group B was first given prednisone in culture medium. After 24 h, TGF- β , IL-23 and IL-6 medium were added to group B culture medium, and cultured for 48 h. The supernatant and cells in culture medium were collected. The content of IL-17 in the supernatant was determined using ELISA kit. The expression level of IL-17 in lymphocytes of each group was determined with immunohistochemistry. For groups C and D, TGF- β , IL-23 and IL-17 were added to each culture medium. After culturing for 48 h, prednisone was added to the culture medium of group C, while group D was treated with blank culture medium. The culture supernatants were collected 24 h later, and the mRNA expressions of IL-17 and IL-23 were assayed with RT-PCR.

Statistical analysis

Measurement data in accordance with normal distribution are expressed as mean \pm standard deviation. Comparison of mean values amongst multiple groups was done with one-way ANOVA, while the SNK test was used for comparison between groups. All statistical analyses were done with SPSS version 19.0 software. Statistical significance was assumed at $p < 0.05$.

RESULTS

Effect of different concentrations of prednisone on mouse lymphocyte proliferation

The MTT assay results showed that prednisone at the concentration of 0.001 M inhibited the proliferation of mouse spleen lymphocytes. The inhibitory effect of prednisone on lymphocyte proliferation at concentrations of 0.01 - 10 μ M was similar in all groups. When the concentration of prednisone was 100 μ M, the proliferation of lymphocytes was significantly inhibited, and the

inhibitory effect was concentration-dependent. These findings are presented in Table 1.

Table 1: Effect of different concentrations of prednisone on mouse lymphocyte proliferation (n = 6)

Group	Prednisone concentration (μM)	Absorbance value (% at 570 nm)
Blank	0	55.6 \pm 30.1
1	0.001	49.3 \pm 9.1*
2	0.01	48.1 \pm 40.6*
3	0.1	47.5 \pm 81.5*
4	1	47.2 \pm 11.1*
5	10	47.0 \pm 39.9*
6	100	34.3 \pm 24.5**
7	1000	26.0 \pm 29.3**

* $P < 0.05$; ** $p < 0.01$, relative to blank

Effect of prednisone on IL-17 level in culture medium (supernatant)

The results in Figure 1 show that IL-17 concentration was markedly reduced in group B, relative to other groups, while IL-17 concentration was markedly downregulated in group C, relative to groups D and A ($p < 0.05$). The results of immunohistochemical staining showed that the expression level of IL-17 was reduced in group B, relative to others. The expression level of IL-17 was highest in group D, and the positive expressions were mainly in the cytoplasmic plasma and interstitial cells of glandular epithelial cells, as shown in Figure 2.

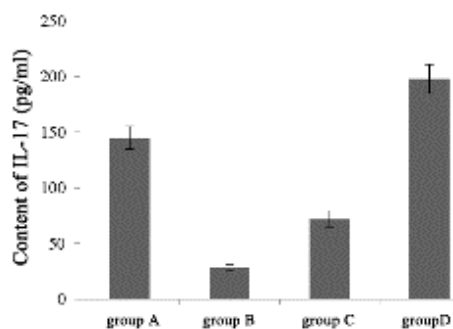


Figure 1: The differences of IL-17 level in the supernatant of culture medium of each of the groups (A - D)

Effect of prednisone on IL-17mRNA and IL-23mRNA levels in mouse spleen lymphocytes

The results revealed markedly lower IL-17 mRNA expression in mouse spleen lymphocytes in group B, relative to other groups ($p < 0.05$). The expression of IL-17 mRNA in spleen lymphocytes was downregulated in C group, when compared to A and D. The expression of IL-23 mRNA between group A and group B were similar ($p > 0.05$). After cytokine stimulation, IL-17 and IL-23 were expressed highly in groups C and D, but IL-

23 mRNA was much lower in group C than in group D ($p < 0.05$). These data are presented in Table 2.

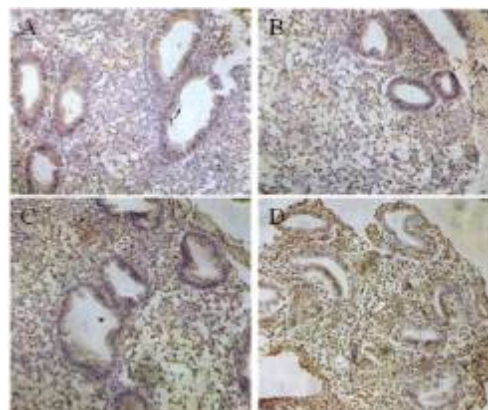


Figure 2: Expression of IL-17 in lymphocytes. A: IL-17 staining of group A as seen under the microscope (immunohistochemistry; $\times 200$); B: IL-17 staining of group B as seen under the microscope (immunohistochemistry; $\times 200$); C: -17 staining of group C as seen under the microscope (immunohistochemistry; $\times 200$); D: IL-17 staining of group D as seen under the microscope (immunohistochemistry; $\times 200$)

Table 2: Effect of prednisone on the expression levels of IL-17mRNA and IL-23 mRNA

Group	IL-17 mRNA	IL-23 mRNA
A	1.055 \pm 0.421* [#]	1.011 \pm 0.052
B	0.194 \pm 0.016	0.563 \pm 0.282
C	0.484 \pm 0.045*	0.840 \pm 0.022
D	2.544 \pm 0.791* [#]	3.162 \pm 1.248 [#]
F-value	15.978	10.424
P-value	0.001	0.040

* $P < 0.05$, compared to group B; # $p < 0.05$, compared to group C

DISCUSSION

Successful pregnancy is the only exception to the principle of allogeneic transplantation and immunology. The mother's immune tolerance to the semi-allografts carrying the father's antigen depends on balance in maternal-fetal interface in the immune microenvironment. Once this maternal fetal immune tolerance process is impaired, immune inflammatory reactions may occur, leading to spontaneous miscarriage, preeclampsia and other pregnancy-related complications [7].

The occurrence of URSA is closely related to imbalance in maternal fetal immune tolerance. The maternal-fetal interface has a complex cell structure with a large number of immune cells. Secreted cytokines such as T cells, NK cells and macrophages constitute a unique cytokine network. The T cells (Th) comprise three sub-groups: Th1, Th2 and Treg, based on their

functions and characteristics. They play immunosuppressive roles *in vivo*, mainly mediating in transplantation tolerance, maternal tolerance and peripheral tolerance [8]. The neoadjuvant T cell, Th17 is found in autoimmune and allergen-specific reactions. It secretes high levels of IL-17, IL-22, TNF- α and other cytokines, resulting in tissue damage by mediating in inflammatory cell infiltration into local tissues, and promoting inflammation and immune rejection [9]. In recent years, several studies have been conducted on Th17 cells regarding their mediation in inflammatory responses, and on T cell balance-mediated immune tolerance.

Prednisone, an adrenal corticosteroid, is widely used in treating diffuse connective tissue disease and immune-related diseases. In the present study, prednisone was used to treat URSA, and its effects on the secretion of IL-17, and on IL-17/IL-23 inflammation axis were investigated. The most important effector of Th17 (IL-17) is widely expressed *in vivo*. It promotes the expression of vascular endothelial growth factor and induces inflammation by activating MAPK and NF- κ B through the innate immune system after binding to the receptor. Besides, IL-17 directly acts on T cells, resulting in induction of autoimmune responses [10,11].

The IL-17R in the decidual tissue of the maternal-fetal interface is the target cell of IL-17. When maternal-fetal immune imbalance occurs, IL-17 acts on the IL-17R receptor on the cell and initiates a series of reactions which may lead to pregnancy failure. Studies have shown that IL-17 levels are much higher in patients with URSA than in women with normal pregnancy. Even in the non-pregnant state, the abortion-prone group has higher IL-17 levels than the non-abortion-prone group [12]. As a pro-inflammatory cytokine, IL-23 activates the secretion of macrophages and monocytes. In addition, IL-23 is closely associated with IL-17 cells. It induces and drives Th-17 to generate IL-17 and IL-6 cells, resulting in inflammatory reactions [13].

It has been shown that IL-23 promotes STAT-3 phosphorylation in decidual immune cells of URSA patients, and increases the secretion of pro-inflammatory factors such as IL-17 and IL-1. However, anti-IL-23 inhibits STAT-3 phosphorylation and up-regulates the release of anti-inflammatory factors such as IL-4 and IL-10. Hence, the IL-17/IL-23 inflammation axis affects the immune balance of Th17 and Treg. The IL-17/IL-23 inflammation axis, a classic axis of inflammation, is implicated in inflammatory gastroenterology and autoimmune meningitis. The IL-17/IL-23 inflammation axis is expected to

be a new target for treating autoimmune diseases and preventing pregnancy failure [14].

The findings from this study have revealed that prednisone, at low concentrations, inhibits the proliferation of lymphocytes and controls the immune response. The inhibition was more obvious with increase in concentration. When TGF- β , IL-23 and IL-6 were used with prednisone before inducing inflammation, the concentration of IL-17 was lowered in group B, relative to the others. The concentration of IL-17 was markedly reduced in group C, relative to D and A when lymphocytes were induced to differentiate into Th17. The expression level of IL-17 mRNA in spleen lymphocytes was much downregulated in group B than in others, and the expression level of IL-17 mRNA in spleen lymphocytes was markedly reduced in group C, when compared to A and D. The expression level of IL-23 mRNA was similar in A and B groups.

There were high upregulations of IL-23 and IL-17 in groups C and D after cytokine stimulation. Group C had lower expression of IL-23 mRNA group D. These results suggest that prednisone inhibits the expression of IL-17 under normal conditions. When immune imbalance occurs, prednisone downregulates IL-17 and IL-23, and inhibits the IL-17/IL-23 inflammation axis, thereby playing a role in immune regulation.

The related mechanism may be that prednisone reduces the production of adhesion molecules such as ICAM-1 and VCAM-1 on the surface of Th17/Th IL-17 cells, blocking their adhesion to the blood-brain barrier cerebral endothelium, thereby inhibiting the ability of Th17/Th IL-17 to enter the nervous system through the blood-brain barrier [15, 16]. Besides, prednisone down-regulates the expression of CD86 in DC cells, increases the antigenic capacity of DC cells, inhibits the maturation of dendritic cells, and suppresses the production of TNF- α and IL-6 by inhibiting the up-regulation of CD86/80 and CD54, thereby blocking the activation of T cells.

CONCLUSION

Prednisone inhibits the proliferation of lymphocytes. When immune imbalance occurs, prednisone suppresses the secretion of IL-17 and down-regulates the expressions of IL-17 and IL-23, thereby regulating immunity by controlling IL-17/IL-23 inflammation axis. The results of this study provide a theoretical basis for treating maternal fetal immune inflammatory response with prednisone.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yuhua Pu designed the study and interpreted the results. Xiaoyan Li, Yuhua Pu collected data and drafted the manuscript. Xiaoyan Li performed the experiments.

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REFERENCES

- 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(6): 443-453.
- Zong S, Li C, Luo C, Zhao X, Liu C, Wang K, Jia W, Bai M, Yin M, Bao S, et al. Dysregulated expression of IDO may cause unexplained recurrent spontaneous abortion through suppression of trophoblast cell proliferation and migration. *Sci Rep* 2016; 6(1): 19916.
- Chen J, Li MJ, Huang QY, Cai JY, Wan JF. Expression of certain cytokines by Th17 cells in peripheral blood and decidua tissues of patients with unexplained recurrent spontaneous abortion. *J Clin Med Pract* 2015; 19(9): 91-93.
- Cai JY, Li MJ. The Research Progress of IL-23 and IL-17 in the Unexplained Recurrent Miscarriage. *Med Recapitulate* 2015; 34: 3461-3463.
- He B, Kuang LJ. Modifying Shoutaiwan combining with prednisone in treating unexplained and recurrent miscarriage. *Chin J Ethnomedicine Ethnopharmacy* 2013; 22(16): 75.
- World Health Organization. Principles of laboratory animal care. *WHO Chron* 1985; 39: 51-56.
- Myhrman A, Rantakallio P, Isohanni M, Jones P, Partanen U. Unwantedness of a pregnancy and schizophrenia in the child. *Br J Psychiatry* 2018; 169: 637-640.
- Xiukui HE, Tao L, Yuan X, Qi YM, Luo XP, Pan BQ. Immunoregulatory effect of soluble Tim-3 on Th1/Th2 differentiation in patients with unexplained recurrent spontaneous abortion and its mechanism. *J Jilin Univ* 2016; 42(6): 3.
- Wang N, Jiang FL. Th1/Th2, Th17/Treg cells and maternal fetal immune tolerance and pathological pregnancy. *Chin J Immunol* 2016; 3(1): 136-139.
- Jiang CR, Wang JB. IL-17 and autoimmune diseases. *J Jining Med Univ* 2015; 13(2): 138-141.
- Jin LJ, Zheng RX, Liu GL, Yang QY. Changes and significance of serum IL-17 and vitamin D levels in children with autoimmune thyroid disease. *Shangdong Med J* 2016; 56(20): 67-69.
- Shi L. Effect of IL-7 on peripheral Th17/Treg, CD4⁺CD8⁻ T cells and pregnancy outcome of unexplained and recurrent miscarriage mouse model. *Matern Child Health Care China* 2016; 31(2): 390-392.
- Yang L, Tang MY, Xu ZZ, Gao AH, Zhang H. Relationship between peripheral Treg/Th17 cells and pregnancy outcomes in the first trimester of patients with unexplained recurrent spontaneous abortion. *Chin J Lab Med* 2017; 40(3): 174-179.
- Faraji F, Rastin M, Arab FL, Kalantari MR, Rabe SZ, Tabasi N, Mahmoudi M. Effects of 1,25-dihydroxyvitamin D3 on IL-17/IL-23 axis, IFN- γ and IL-4 expression in systemic lupus erythematosus induced mice model. *Iran J Basic Med Sci* 2016; 19(4): 374-380.
- Bai H, Liu F. Efficacy of modifying Xiaokangtang combining prednisone in treating immune infertility. *Shaanxi J Trad Chin Med* 2015; 1(10): 1279-1280.
- Zhang S, Shao SY, Song XY, Xia CY, Yang YN, Zhang PC, Chen NH. Protective effects of Forsythia suspense extract with antioxidant and anti-inflammatory properties in a model of rotenone induced neurotoxicity. *Neurotoxicol* 2016; 52(2): 72-83.