

The effect and different expressions of t lymphocyte before and after immunotherapy in patients with recurrent spontaneous abortion.

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Abstract

Objective: To investigate the different serum expressions of CD3+, CD4+ and CD8+ in the peripheral blood among the patients with Recurrent Spontaneous Abortion (RAS) before and after immunization therapy and to evaluate the clinical effects and pregnancy outcomes.

Methods: 157 RAS patients with negative blocking antibody in our hospital were enrolled into the study and treated with immunity therapy from September 2015 to August 2017. The blocking efficiency of T lymphocytes (CD3+, CD4+ and CD8+) in peripheral blood of patients with the lymphocyte of patients' husband (or unrelated healthy male) as antigen target were measured by double fluorescent marker flow cytometric analysis technique.

Results: After treatment, the serum blocking efficiency of CD3+, CD4+, CD8+ were higher than before treatment, $P < 0.05$. And the serum blocking efficiency of CD3+, CD4+, CD8+ in 37 RAS patients with positive blocking antibody after treatment were higher than before treatment or 121 RAS patients with negative blocking antibody, $P < 0.05$. Besides, there were 18 RAS patients (14.88%) who had negative blocking antibody after treatment with successful pregnancy and 12 RAS patients (33.33%) who had positive blocking antibody after treatment with successful pregnancy.

Conclusion: Active immunotherapy can improve the blocking efficiency of CD3+, CD4+ and CD8+ in peripheral blood of recurrent spontaneous abortion patients there for helping pregnancy, which would be recommended for the clinical used as an effective treatment.

Keywords: Recurrent spontaneous abortion, Immunization therapy, Blocking antibody, T lymphocyte, Pregnancy outcome.

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Introduction

Recurrent Spontaneous Abortion (RSA) is refer to patients who lose pregnancy outcome or fetus naturally over 28 w three times or more than three times [1,2]. In recent years, with the openness of two-child policy, environmental pollution is getting more and more worse, life and work pressure is becoming more and more heavy, the incidence rate of RSA in gradually increases [3]. From the immunology perspective, RSA can be divided into immune RSA and non-immune RSA. Non-immune RSA has relations with genetic factors, infection, reproductive structure or abnormal hormone secretion level and so on [4]. Immune RAS may be caused by short of parent relevant antibody or blocking antibody, it caused by resistance produced by embryo paternal line antibody [5]. Natural T regulatory cell (nTreg) plays an important role in partial the same kind immune RAS patients during abortion process. nTreg produce CD3+, CD4+ and CD8+T lymph cells through activation, thus, producing immune effects on mother-fetus tolerance [6]. Some RAS patients lack of blocking antibody, so it cannot produce tolerance for pregnancy products, thus, causing mother immune system produce immune attack for embryo paternal line antibody, finally inducing abortion.

Immunization therapy is the common treatment methods of some RAS. The purpose of this study is to explore the diagnostic and treatment value of T lymph cell activation rate in clinical RAS.

Materials and Methods

Clinical data

Ethical approval was given by the medical ethics committee of The Second Affiliated Hospital of Shaanxi University of Chinese Medicine with the following reference number: 2015016. This study selected 157 RAS patients with immunization treatment in reproductive center in our hospital from September, 2015 to August, 2017. The age was from 20 to 43 y old. The average age was 33.27 ± 6.34 y old. All patients were given blocking antibody detection, it was negative.

Inclusive criteria: First, natural abortion (pregnancy within 28 w) equal to or more than three times; second, blocking antibody was negative; third, chromosome of maternal and paternal lines were normal; fourth, reproductive system

structure in mother had no abnormality; fifth, the ovulation was normal; sixth, the sperm was normal; seventh, indexes, such as mother endocrine, blood routine, urine routine, liver and kidney function, were normal; eighth, infections, such as mycoplasma, chlamydia, TP and so on were excluded; ninth, ACA, EMAB, ANA, anti-sperm antibody all were negative; tenth, patients or families signed consent form.

Exclusive criteria: First, patients who not met the criteria above; second, patients with abnormal chromosome; third, patients with reproductive system diseases; fourth, patients with autoimmune diseases or malignant tumor; fifth, patients with emotional disorder; sixth, patients with liver and kidney dysfunction; seventh, patients with poor compliance and so on.

Treatment strategies

All subjects were willing to accept treatment. This study collected the 50 ml third elbow vein blood of patients' husband and healthy male without kinship. It was placed in anticoagulative tube, given centrifugation. The supernatant was discarded. The SN was added. All process was conducted under the situation of sterility. The lymph cell concentration was regulated to $3^{-5} \times 10^8/\text{ml}$. 1 ml lymph cell suspension was injected into lateral part of forearm of patients about six to eight times, then we observed for 30 min. Skin swelling, bubble, hematoma and so on should be given corresponding management. If there were no reactions, the treatment should be continued. They were given one time every two week. Three times one course. They were given once every six weeks. For negative patients with examination, the second course should be continued until to the third month after pregnancy [7].

Observation indexes

First, this study collected peripheral blood when detected positive immune treatment. This study used double fluorescent flow cytometry to detect blocking efficiency of CD3+, CD4+, CD8+ patients. If there were any negative value, the blocking antibody was negative, it can be included treatment area. Second, effects evaluation: it not transformed into positive (before and after treatment, a certain detection index was still negative), transformed into positive (a certain detection index was negative before treatment, positive after treatment). Third, pregnancy results: pregnancy failure (HCG by blood examination before and after treatment were still negative, HCG was positive after treatment, but it had abortion again

during maintaining treatment), success pregnancy (HCG was positive by blood detection before treatment, positive after treatment, there was no abortion after three months of maintaining treatment).

Statistical management

This data used SPSS 21.0 to do t-test and χ^2 test. Measurement data used paired t test to do data comparison between two groups; enumeration data used χ^2 test or Fisher exact test. $P < 0.05$, there were statistical differences.

Results

Changes of T lymph cell blocking efficiency before and after treatment

Compared with before treatment, blocking efficiency of CD3+, CD4+, CD8+ cells of patients after treatment improved obviously, there were statistical differences, $P < 0.05$, Table 1).

Table 1. Comparison of peripheral blood CD3+, CD4+, CD8+ value of RAS patients before and after treatment ($\bar{x} \pm s$).

Detection time	CD3+ (%)	CD4+ (%)	CD8+ (%)
Before treatment	42.13 ± 11.44	31.17 ± 4.84	21.68 ± 3.57
After treatment	56.74 ± 8.29	38.03 ± 7.66	25.14 ± 4.47
t	5.453	3.691	3.996
p	0.017	0.024	0.019

Comparison of T lymph cell blocking efficiency of positive and negative before and after treatment

After treatment, 157 RAS patients were given blocking antibody reexamination, blocking antibody of 36 cases were positive, other 121 patients were negative. Compared with before treatment, CD3+, CD4+, CD8+ cell percentage of patients in positive group increased obviously, there were statistical differences, $P < 0.05$; CD3+, cell percentage in negative group increased obviously, there were statistical differences, $P < 0.05$. CD4+ and CD8+ cell percentage had no changes, $P > 0.05$. In addition, after positive immune treatment, CD3+, CD4+ and CD8+ cells percentage higher than negative group obviously, there were statistical differences, $P < 0.05$, Table 2).

Table 2. Comparison of T lymph cell blocking efficiency of positive and negative before and after treatment ($\bar{x} \pm s$).

Detection time	Positive (n=36)			Negative (n=121)			t1	t2	t3	p1	p2	p3
	CD3+ (%)	CD4+ (%)	CD8+ (%)	CD3+ (%)	CD4+ (%)	CD8+ (%)						
Before treatment	44.46 ± 10.78	31.82 ± 8.13	21.73 ± 3.26	41.05 ± 9.48	30.01 ± 5.37	20.99 ± 3.04	0.874	0.455	1.026	0.531	0.768	0.242
After treatment	60.21 ± 12.74	39.56 ± 6.81	26.17 ± 5.36	52.33 ± 8.47	32.47 ± 7.26	22.53 ± 3.51	3.875	4.263	4.098	0.023	0.020	0.021

t	7.634	3.967	4.228	2.482	1.564	1.626	-	-	-	-	-	-
p	0.005	0.025	0.020	0.047	0.051	0.050	-	-	-	-	-	-

Comparison of pregnancy rate of patients in positive and negative group

After treatment, 12 patients had pregnant successfully in positive group. The successful pregnancy rate was 33.33%, higher than patients in negative group, there were statistical differences, P<0.05, Table 3).

Table 3. Influences of CVVH in immune cells in peripheral blood of patients (n, %).

Group	n (case)	Successful pregnancy	Pregnancy failure	χ^2	p
Positive patients	36	12 (33.33)	24 (66.67)	3.769	0.028
Negative patients	121	18 (14.88)	103 (85.12)		

Discussion

In the 1970s, Rocklin find blocking antibody in serum of pregnant women firstly, belongs to IgG antibody of the same category produced by trophocyte, it produced by paternal line stimulating maternal line system. It can combine with antigen competitively to avoid mother immune cell recognition and kill paternal antigen [8]. With the in-depth study, more and more scholars find that lacking blocking antibody can cause abortion. Therefore, for these immune RAS patients, it is suggested that initiative immune treatment in clinic [9]. Effects and mechanism of initiative immune treatment is to improve mother blocking antibody positive rate, give subcutaneous injection of the third individual lymph cells of husband or healthy male without kinship to RAS patients, it can promote immune tolerance ability by stimulating mother to produce immune response. Results of this study show that initiative immune treatment can promote blocking rate of RAS patients. After immunization treatment, CD3+, CD4+, CD8+ cell expression rate in serum of patients increase obviously. The possible effects and mechanism of it is stimulating immune system of patients by introducing exogenous antigen passively. It can produce autoimmune regulation, activate mother regulatory T cells, and promote number of T cells in peripheral blood. Therefore, monitoring T cell lymph expression during initiative immune treatment process in RAS patients is quite important.

T lymph cells is the most important immune cell group of body, can be divided into regulatory T lymph cells, assistant T lymph cells and cellular toxic T lymph cells. nTreg can activate CD4+ and CD8+ T lymph cells by signal system induced by TCR, thus, inducing pregnancy immune tolerance ability. If the percentage of nTreg in peripheral blood is too low, the immune tolerance of mother on pregnancy outcome or fetus will decrease, causing abortion [10,11]. CD4+T lymph cells belong to assistant T lymph cells, it can regulate cellular immunity and

liquid immunity by secreting some cellular factors. CD4+T lymph cells have cellular toxic T lymph cells and inhibiting T lymph cells, it participates in inhibiting immune response by specificity recognizing antigen of target cell surface [12].

From the immunology perspective, in fact, pregnancy process is similar to organ transplantation. Successful pregnancy or not depends on maintaining conditions of immune state between mother and fetus. Embryo itself has paternal line antigen. If there are rejection reaction on pregnancy outcome or fetus by mother, will cause pathological pregnancy [13,14].

Blocking antibody can induce mother induce immune tolerance, promote increase of regulatory T lymph cells. When they pregnant again, it can reduce immune attack on embryo homologous semi graft antigen, avoid abundant immune response, so it can protect embryo in order to improve pregnancy success rate [15]. There are still arguments on relations between blocking antibody and pregnancy outcome in medical field. Studies of Jablonowska et al. and Bouvier et al. [16,17] think that blocking antibody has no correlations with pregnancy outcome. But there are many studies demonstrate that blocking antibody positive rate and recurrent pregnancy rate of RAS patients after lymph cells initiative immune treatment increase obviously [18,19]. This study finds that pregnancy success rate of blocking antibody positive patients higher than negative patients obviously, there were statistical differences, P<0.05. It may be caused by individual differences in blocking antibody. Therefore, statistical results have deviation.

In conclusion, after initiative immune treatment, T lymph cells activation rate in RAS patients increase obviously. Recurrent pregnancy rate of blocking antibody positive patients improve, it shows that monitoring positive rate of blocking antibody during initiative immune treatment of RAS patients closely has significant clinical value in predicting pregnancy outcome.

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