



Clinical observation of lymphocyte active immunotherapy in 380 patients with unexplained recurrent spontaneous abortion



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ABSTRACT

Purpose: This study aims to investigate the clinical curative effect of lymphocyte active immunotherapy (LAI) on unexplained recurrent spontaneous abortion (RSA).

Methods: A total of 749 RSA patients who received medical service in our hospital from October 2009 to June 2013 were enrolled into this study. These patients were randomly divided into two groups: LAI group (treatment group) and routine progesterone for maintenance tocolysis group (control group). A comparative analysis on the pregnancy outcomes in these two groups was conducted.

Results: Abortion rate was significantly lower in the LAI group than in the control group ($P < 0.05$). Furthermore, pregnancy success rates were 89.7% and 32.2% in patients who received LAI and routine progesterone for maintenance tocolysis, respectively, and the difference was statistically significant ($P < 0.05$).

Conclusion: Our analysis suggested that LAI can treat RSA effectively and has an excellent clinical effect. Furthermore, the detection of blocking antibodies showed a positive prediction on pregnancy outcome.

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1. Introduction

Recurrent spontaneous abortion (RSA) clinically refers to two or more consecutive spontaneous abortions [1]. The risk rate in patients with a history of two spontaneous abortions is 24.4% in their next pregnancy, while the risk rate in patients with a history of three or more spontaneous abortions is 33.1% in their next pregnancy [2]. The number of RSA cases accounts for 0.2–0.8% of the total number of pregnancies. RSA occurs due to a variety of reasons, and 40–70% of these reasons remain unknown. With the development of medicine in recent years and the advance of reproductive immunology studies, some scholars have considered that >40% of unexplained RSA is associated with immune factors [3–5]. There are many reports on LAI in patients with unexplained RSA using lymphocytes obtained from the patient's husband or a healthy irrelevant person [6]. However, this treatment scheme and effect remain controversial. In this study, 380 RSA patients who received LAI in the Reproduction Medicine Department of our hospital from October 2009 to June 2013 were analyzed, in order to investigate the clinical curative effect of LAI on unexplained RSA.

2. Materials and methods

2.1. General information

Data of 749 RSA patients who received medical service in our hospital from October 2009 to June 2013 were retrospectively analyzed. The age of these patients ranged between 24 and 36 years old (average age, 32.6 ± 2.4 years old). All patients met the following criteria: patients who had ≥ 2 abortions during early pregnancy. Furthermore, chromosome karyotypes of the couples were normal, and patients had no abnormal reproductive tract anatomy. Thyroid, endocrine functions and the insulin level were normal. In addition, TORCH IgM was negative, antiphospholipid and antinuclear antibodies were negative, and the coagulation function was normal. Furthermore, cervical secretion was negative. The couples were also found to be negative for hepatitis B, hepatitis C, syphilis, AIDS or other infectious diseases. In addition, blocking antibodies was not detected. Semen examination of the patient's partner showed normal. Among patients with normal results in the above examinations, 380 patients voluntarily received LAI and provided a signed informed consent (treatment group). The remaining 369 patients did not accept LAI and only accepted routine progesterone for maintenance tocolysis (control group).

Unexplained RSA has also been identified as alloimmune recurrent abortion. The underlying mechanism is suggested to be that the mother presents a low immune response to the fetus due to abnormal antigen recognition to the paternal antigen, which results in the lack of maternal blocking antibodies and other cellular and humoral immune

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abnormalities. This would subject the embryo to attack from the abnormal immune system, causing recurrent abortion. Its pathogenesis includes the following: (1) Leukocyte antigen and alloimmune RSA: Substantial evidences have suggested that the RSA couple had a significantly higher probability to have common HLA, compared with normal controls. Furthermore, it was found that antigens associated with recurrent abortion were mainly expressed on the HLA-DR and DQA1 loci. A large number of studies have revealed that RSA patients have susceptibility genes and susceptibility gene haplotypes, and the genetic loci varied in different races. The gene products encoded by these susceptibility genes and susceptibility gene haplotypes may exist in the HLA complex, and cause the mother to have a low response to the embryo. HLA-G immune regulation forms the balance of Th1/Th2 cytokines, and promotes this balance to move towards the Th2, which is beneficial to the pregnancy. (2) Disturbance of cellular immunity: This is mainly caused by natural killer (NK) cells and T cells. RSA patients show abnormal cell subsets of the immune system in their bodies. After LAI, the number of CD4 + CD25 + Treg cells significantly increased compared with pre-treatment, and IL-2 activity significantly decreased. This promotes immune protection of the embryo and suppresses immune damage to the embryo, which is conducive to the success of the pregnancy. (3) Th1/Th2/Th17 and Treg regulatory cytokine balances: The number of T regulatory cells increased in normal pregnant women, and declined in RSA patients. (4) Trophoblast-lymphocyte cross-reactive antigens (TLX): Blastocyst implantation and fetal development require the immune identification of the mother to TLX, while embryonic trophoblast cells exhibit a low identification to TLX in RSA patients.

The serum blocking antibodies in normal pregnant women mainly include (1) warm-B cell antibody, (2) cold-B cell antibody, (3) specific antibody, (4) anti-TLX antibody, (5) anti-Fc receptor antibody, and (6) antipaternal complement-dependent antibody.

ELISA method was used to detect blocking antibodies in the blood of patients, which is easy to master, affordable and can easily be accepted by doctors and patients. All medicine studies are performed for guidance in clinical work. Practice has proven that LAI for unexplained recurrent abortion patients have achieved good results. In our work, more patients began to receive LAI. The implantation rate and birth rate significantly increased in patients who have undergone IVF-ET and suffered from repeat failure of implantation. This treatment method is worthy of clinical popularization and application.

2.2. Research methods

Groupings: Patients in the treatment group were further divided into three subgroups according to the different sources of lymphocytes and injection timing. Group A: conventional scheme, the lymphocyte came from the husband, patients were injected every 2–3 weeks, four treatments were performed before pregnancy, and three treatments for strengthening were performed after pregnancy. Group B: emergency scheme, RSA patients who had been pregnant and urged LAI were injected once a week, four consecutive treatments were performed, and blocking antibodies were determined. If blocking antibodies were positive, the treatment was terminated, while if blocking antibodies were negative, the treatment was continued. Group C: alternative scheme, the patient's spouse could not provide lymphocytes due to blood-borne infectious diseases or other reasons. Lymphocytes from irrelevant healthy men were used for treatment, and the method used was the same as that in group A.

Lymphocyte active immunotherapy method: Before treatment, five items of hepatitis B, hepatitis C antibody, syphilis, and HIV of lymphocyte donors (patients' spouses or healthy irrelevant men) were detected. LAI was performed when all examinations revealed normal results. Under sterile conditions, 25–30 ml of venous blood was withdrawn from the patient's spouse or healthy irrelevant men, the blood samples underwent anticoagulation by heparin, lymphocytes were extracted from the lymphocyte separation liquid by Ficon density gradient centrifugation, and washed twice with physiological saline to prepare cell suspension. The lymphocytes were isolated using a special lymphocyte separation liquid (Density-separating liquid, from The Tianjin Haoyang bioproduct company). Then, the supernatant was discarded. Lymphocytes were diluted again with 0.8–1.0 ml of saline. Each time, approximately $1 \times 10^7 - 3 \times 10^7$ lymphocytes were obtained. Intracutaneous injections were given at four to six points on the patient's forearm, and approximately 0.2 ml was injected at each point. One treatment was given every 2–3 weeks, and four treatments were given before pregnancy as a treatment course. Two weeks after the termination of the treatment course, blocking antibodies were determined. If results were positive, patients were encouraged to undergo pregnancy within six months; while if results were negative, the next course of LAI was carried out. After pregnancy, three LAIs were given for strengthening once every two weeks.

Blocking antibody determination using enzyme-linked immunosorbent assay (ELISA): The blocking antibody detection kit was produced by Lambda (USA) and was provided by Beijing Suoao Biotechnology. Approximately 2.0–3.0 ml of the patient's peripheral venous blood was extracted, serum was isolated by centrifugation (4000 rpm), and blocking antibodies were determined by ELISA. A reagent reaction pore that turns blue was considered positive, while colorless was considered negative.

Diagnosis criteria of successful pregnancy: pregnancy occurs after blocking antibodies turn positive, pregnancy duration is >20 weeks, and ultrasonic examination suggests that the fetus develops well in the uterus.

2.3. Statistics analysis

Data was analyzed using statistical software SPSS 11.5. Data were expressed as mean \pm standard deviation (SD), and inter-group comparison was carried out by Chi-square test. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of general information

Differences in age, number of abortions, and abortion recurrence rate between the treatment and control groups were not statistically significant ($P > 0.05$). In the treatment group, 341 of 380 patients had maintained their pregnancy for >20 weeks, with a success rate of 89.7%. In the control group, 369 patients received routine maintenance tocolysis. Among these patients, 119 patients developed successful pregnancy, with a success rate of 32.2%. The difference in success rate between these two groups was statistically significant ($P < 0.05$, Table 1).

Table 1
Comparison of general information [$(\bar{x} \pm s)$, n(%)].

Groups	Cases	Age	Number of abortions	Abortion recurrence rate	Success rate
Treatment group	380	29.4 \pm 3.4	3.5 \pm 2.6	39(10.3)	341(89.7)
Control group	369	27.7 \pm 3.5	3.6 \pm 2.2	250(67.8)	119(32.2)

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