

Effect of eliminating intermittent white blood cells on immunology and cellular factors of systemic lupus erythematosus

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Background: SLE is treated currently by multiple immunosuppression, but side-effects are obvious after long time administration. This study was to observe changes in T, B cells, NK cells and IL12 in patients with systemic lupus erythematosus (SLE) before and after treatment of eliminating intermittent white blood cells and to probe the mechanism of this treatment.

Methods: In 23 patients with SLE, 5 were male and 18 female, with an average age of 15.78 ± 5.40 years. These patients accepted treatment of eliminating intermittent white blood cells. The expressions of $CD19^+$, $CD3^+$, $CD4^+CD8^+$, $CD4^+/CD8^+$, $CD(15+56)^+$ were tested by flow cytometry before and after the treatment. The amounts of immunoglobulins, IgM, IgG, IgA in periphery blood were measured separately by immunoradiometric analysis before and after the treatment. The level of IL12 was detected by ELISA. Twenty volunteers served as controls.

Results: The expression of $CD19^+$ in the patients increased markedly before the treatment. Statistical significance was noted in the control group and the patient group after the treatment ($P < 0.01$ or $P < 0.001$). The expression of IgM and IgG increased markedly before the treatment. Statistical significance was seen between the control group and the patient group after the treatment ($P < 0.01$ or $P < 0.001$). The expressions of activated $CD3^+$ and $CD8^+$ increased markedly in the patients with SLE before the treatment ($P < 0.05$) ($P < 0.01$ or 0.001) respectively. The expressions of $CD3^+$ and $CD4^+$ decreased markedly ($P < 0.01$ or $P < 0.05$) ($P < 0.001$) respectively. The ratio of $CD4^+$ to $CD8^+$ decreased markedly ($P < 0.01$). The expression of $CD3^+$ after the

treatment decreased more remarkably in the patients with SLE than in the control group ($P < 0.05$). The changes in the expression of $CD(15+56)^+$ suggested that the expression of $CD(15+56)^+$ increased markedly before the treatment. Significant statistical difference was observed in the patient group and the control group after the treatment ($P < 0.05$ or $P < 0.001$). The expression of IL12 in the patients with SLE decreased, but it decreased more significantly than in the control group before the treatment ($P < 0.05$ or $P < 0.01$).

Conclusions: Since patients with SLE have the disturbances in T, B cellular immunology and NK cells, IL12, the treatment of eliminating intermittent white blood cells has regulatory effects on T, B cells immunology and NK cells, IL12 in the patients with SLE.

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Key words: SLE; immune; NK cell; IL12; eliminating intermittent white blood cells

Introduction

Systemic lupus erythematosus (SLE) is one of the autoimmune diseases with unknown causes. The disease is treated currently by multiple immunosuppression, but side-effects are obvious after long time administration. Some immunosuppressants are too expensive to be used in patients with SLE; therefore, researchers are seeking new ways for the treatment of SLE with less side-effects. To increase the efficiency of the treatment, eliminating intermittent white blood cells (WBCs) has been used in patients with SLE at our department since January 2000. The result of this treatment has been significant.^[1,2] To probe the mechanism of the treatment, this study focused on the measurements of T, B cell immunity, interleukin 12 (IL12), and NK cells in patients with SLE before and after the treatment.

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Methods

Subjects

Twenty-three patients with SLE were admitted to our hospital from January 2000 to December 2003. Among them, 5 were male and 18 female. Their average age was 15.78 ± 5.40 years. The diagnostic criteria were based on *Zhu Fu-tang Practical Pediatrics*, 6th edition.^[3] Twenty volunteers who had normal physical findings as well as normal peripheral blood routine, and liver, renal functions served as controls.

Observation

All 23 patients accepted the treatment of eliminating intermittent white blood cells, using a disposable white blood cell separation device. 200-400 ml blood was taken each time (1-2 times each week; if conditions are stable, 1 time a month), and 4 ml venous blood was taken respectively before and after the treatment of eliminating white blood cells for the assessment of changes in IgG, IgA, IgM, CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺, CD19⁺, CD(16+56)⁺ and IL12 in addition to multiple immune indexes and cellular factors. At meantime, 20 samples were taken from the control group consisting of 10 males and 10 females with an average age of 11.42 ± 3.51 years.

Measurement

Lymphocytic subgroups were measured by a FACS caliber flowing cytometer (BD company, USA) with a testing kit of simulestTMIMKPlus (BD company). Immunoglobulins were tested by DADE BehringBNProsel, and IL12 level in plasma was assessed by double antibody ELISA. Immunoglobulins IgG, IgM and IgA were measured by the immunoradiometric assay. Whole blood was stained by the direct double

immunofluorescent method and lymphocytic subgroups were measured by a flowing cytometer by adding 20 μ l fluorescent labeling monoclonal antibody into 100 μ l blood with anti-agglutinant, and reacted for 20 minutes at 4°C. Subsequently, it was centrifuged for 5 minutes at a speed of 1000 r/min while discarding supernatant and washing by PBS solution for two times, and put into a FACS caliber flowing cytometer for regular analysis. With a wavelength of 488 nm, 10⁴ cells were collected from each sample for the measurement of IL12. The sample was centrifuged at a speed of 3000 r/min in a high speed centrifuge. Supernatant was kept, and the sample was placed in a -78°C freezer for use.

Statistical analysis

All data were expressed by mean \pm standard deviation (mean \pm SD); comparison between the groups was made by Student's *t* test.

Results

Signs and symptoms of the 23 patients were improved significantly after the treatment. The immune indexes and the level of IL12 also changed.

Changes in B cell immunity

CD19⁺ before the treatment in the 23 patients was increased more significantly than that after the treatment in the two groups ($P < 0.01$ or $P < 0.001$). The expression of IgM and IgG before the treatment was increased more significantly than that after treatment in the two groups ($P < 0.01$ or $P < 0.001$). The level of IgA increased before the treatment was not significantly different from that after the treatment or from that of the control group. These indexes decreased more

Table 1. Results of CD19⁺ and immunoglobulins IgG, IgM and IgA (mean \pm SD)

Group	<i>n</i>	CD19 ⁺ (%)	IgG (g/L)	IgM (g/L)	IgA (g/L)
Before treatment	23	23.67 \pm 13.85*** $\Delta\Delta$	20.22 \pm 8.01*** $\Delta\Delta$	1.62 \pm 0.72*** $\Delta\Delta$	1.86 \pm 0.73*
After treatment	23	14.61 \pm 8.97	9.25 \pm 3.38	1.11 \pm 0.62	1.60 \pm 0.63
Controls	20	12.05 \pm 5.82	8.76 \pm 2.45	1.01 \pm 0.43	1.21 \pm 0.55

Compared with control group, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$; compared with those after treatment, Δ : $P < 0.05$, $\Delta\Delta$: $P < 0.01$, $\Delta\Delta\Delta$: $P < 0.001$.

Table 2. Results of CD3⁺ and activated CD3⁺ (mean \pm SD)

Group	<i>n</i>	CD3 ⁺ (%)	Activated CD3 ⁺ (%)
Before treatment	23	63.37 \pm 11.66*** Δ	15.08 \pm 3.41* Δ
After treatment	23	70.48 \pm 7.96*	8.67 \pm 4.25
Controls	20	78.35 \pm 8.56	6.08 \pm 3.45

Compared with controls, *: $P < 0.05$, ***: $P < 0.001$; compared with those after treatment, Δ : $P < 0.05$.

Table 3. Results of CD4⁺, CD8⁺, CD4⁺/CD8⁺ (mean±SD)

Group	n	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
Before treatment	23	34.61±10.75***△△△	42.58±13.21***△△	0.72±0.42**△△
After treatment	23	47.70±13.31	32.39±9.25	1.34±0.45*
Controls	20	52.50±8.75	31.15±7.25	1.40±0.35

Compared with controls, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$; compared with those after treatment, △△: $P<0.01$, △△△: $P<0.001$.

Table 4. Results of CD(16+56)⁺ (mean±SD)

Group	n	CD(16+56) ⁺ (%)
Before treatment	23	7.57±4.10***△
After treatment	23	11.65±8.20
Controls	20	15.76±7.75

Compared with controls, ***: $P<0.01$; compared with those after treatment, △: $P<0.05$.

Table 5. Results of IL12 (mean±SD)

Group	n	IL12 (Pg/ml)
Before elimination of WBC	28	50.21±30.16**△
After elimination of WBC	42	65.96±30.10*
Controls	20	169.90±90.02

Compared with controls, **: $P<0.05$; compared with those after treatment, △: $P<0.05$.

significantly than those of the control group after the treatment, but there was no significant difference (Table 1).

Changes in T cell immunity

Comparison of the two groups showed the expression of activated CD3⁺ increased significantly in the patients with SLE before the treatment ($P<0.05$). The expression of CD8⁺ increased significantly ($P<0.01$ or $P<0.001$), but the expression of CD3⁺ decreased significantly ($P<0.001$ or $P<0.05$). The expression of CD4⁺ and the ratio of CD4⁺/CD8⁺ decreased significantly ($P<0.001$) ($P<0.01$) respectively. The expression of CD3⁺ and the ratio of CD4⁺/CD8⁺ in the patients with SLE after the treatment decreased more significantly than in the controls ($P<0.05$). The expression of activated CD3⁺, CD4⁺ and CD8⁺ was not obviously different from that of the controls (Table 2).

Changes in the expression of CD(16+56)⁺

The expression of CD(16+56)⁺ increased significantly before and after the treatment in the controls ($P<0.05$ or $P<0.001$) (Table 4).

Changes in IL12

The expression of IL12 decreased more significantly before the treatment in the patients with SLE than in the controls ($P<0.05$ or $P<0.01$) (Table 5).

Discussion

SLE is characterized by production of autoimmune antibody and abnormal intracellular and extracellular immunological reactions. Patients with SLE have dysfunction of B lymphocytes.^[4] As a pancreatic

protein in the Tg-SF family, CD19⁺ is a specific symbol of B cell and a member of the CR2 complex. It can regulate the activation and proliferation of B cell, and participate in the signal transmission of B cell. The abnormality of T cell subgroup may play an important role in inducing the disease.^[5,6] The decreased number and dysfunction of T supply cells cause hyperimmune reaction, and the VV subgroup of CD8⁺ cell play an inhibitory role. Once the CD8⁺ VV subgroup is activated, CD8⁺ cell as a whole can not express its inhibitory function completely,^[7,8] thus leading to the disturbance of the immune system.

NK cells are also important immune regulating cells. The specific symbols of human NK cell surface are CD16 and CD56. NK cells mainly regulate the body immune system by releasing cellular factors which can inhibit the division and proliferation of B cells. They also regulate T cell mediated immunity.^[9] In this study, the expression of CD(16+56)⁺ decreased more significantly in the patients with SLE before the treatment than after the treatment, suggesting the reduced regulation to T, B cells by weakened NK cells. Hence unbalances occurred in T, B, NK cells, resulting in the disturbance of the immune system in patients.

Th1 cellular level and elevated Th2 cellular level were abnormal in the patients with SLE in this group. IL12 is a dimer consisting of two polypeptide chains bonded covalently with one with 3500 aa, and the other with 4000 aa. It is produced after stimulation of antigens by monocytes, macrophages, B cells and other accessory cells.^[10] IL12 plays an important role in B cell mediated immune reaction,^[11,12] it transforms Th cells to Th1 cells, promotes the proliferation of T cells and NK cells, induces the production of IFN- γ , and enhances the division of CTL. The abnormal cellular factors of SLE include mainly reduction of Th1

cellular factor. Patients with SLE often have a reduced level of IL12.^[13,14] In this study, the expression of IL12 decreased more significantly before the treatment in the patients with SLE than in the control group.

Previously, eliminating intermittent WBCs was only used in the treatment of hematological diseases and in blood transfusion. Some researchers reported plasma replacement in the treatment of rheumatic diseases,^[15] but high medical cost and complications such as disturbance of electrolytes and infections prevented patients with SLE from this treatment. In recent years, stem cell transplantation has been used in the treatment of SLE, but it is expensive and restricted by the source of donors. We have been using the treatment of eliminating intermittent WBC in the treatment of patients with SLE since 2000. The clinical effect of the treatment is satisfactory,^[1] and side-effects such as lower proteinemia, disturbance of electrolytes, infections, and hemolysis have been controlled. The clinical signs and symptoms of patients can be improved significantly after the treatment of eliminating intermittent WBCs. Immune index and cellular factor IL12 have been improved simultaneously, elucidating that treatment of eliminating intermittent WBCs regulates T cells, B cells, and IL12 in patients with SLE.

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Contributors: JJJ proposed the study and wrote the draft. FF analysed the data. GLF and CRH provided advices on medical aspects.

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