Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Clinical efficacy and mechanism of lymphoplasma exchange in the treatment of Guillain-Barre syndrome

M-C. Luo[1,2], W-F. Wang[3], W-F. Yin[4], Y. Li[5], B-J. Li[6], W-W. Duan[5], Q-M. Zeng[1], Y-B. Luo[1], X-S. Yang[1], H. Yang[1*]

[1] Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China
[2] Department of Geriatrics, Xiangya Hospital Central South University, Changsha, Hunan, China
[3] Department of Neurology, People's Hospital of Liaocheng, Liaocheng, Shandong, China
[4] Department of Neurology, Xiangya Second Hospital, Central South University, Changsha, Hunan, China
[5] Department of Neurology, First Hospital of Changsha, Changsha, Hunan, China
[6] Department of Blood Transfusion, Xiangya Hospital, Central South University, Changsha, Hunan, China

Correspondence to: jingji342yonggao9@163.com

Received August 4, 2017; **Accepted** October 18, 2017; **Published** October 31, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.10.17

Abstract: Guillain-Barre syndrome (GBS) is an autoimmune disease of the nervous system and is the most common acute polyneuropathy. Both cellular and humoral immunity are believed to be involved in the pathogenesis of GBS, and various types of activated CD4+ T cells are thought to orchestrate the onset and progression of GBS. Lymphoplasma exchange (LPE) filtering out activated lymphocytes while exchanging plasma has been used for GBS treatment for years. However the treatment is still not yet optimal. In order to assess the efficacy of this treatment, we evaluate the effect of LPE and determine the appropriate frequency of LPE treatments for GBS patients through comparing the neurological deficit scores and the changes in related immunology indicators of GBS patients before and after LPE treatment. Twenty-four patients with GBS who received LPE were evaluated for immunologic indicants before treatment, on the second day, and the fourth day after the treatment. The immunoglobulin complement and $CD4^+$ T lymphocyte subsets were tested by flow cytometry. The patients' Medical Research Council sum scores were increased from 25.7±10.4 up to. 36.7±10.4 (P=0.019) and their Hughes scores decreased from 3.7±0.76 to 3.1±0.73 (P=0.027) at 7 days after LPE. In the peripheral blood from patients received LPE treatment, the levels of immunoglobulin, complement, monocytes and fibrinogen were significantly reduced. The percentages of Th1 and Th17 cells in the CD4+ T lymphocyte subsets were significantly decreased, whereas the Th2 and Treg cells were increased in patients after treatment. The changes in $CD4^+$T lymphocyte subsets were correlated with patient MRC score changes. Our data indicate that LPE is effective in treating GBS patients by directly removing immunoglobulin, complement, monocytes, and fibrinogen as well as regulating lymphocyte subsets in the peripheral blood.

Key words: Lymphoplasma exchange; Guillain-Barre syndrome; Clinical efficacy; T lymphocyte subsets; Percentage of monocytes; Fibrinogen.

Introduction

Guillain-Barre syndrome (GBS) is an autoimmune disease of the nervous system, and the most common acute polyneuropathy. GBS affects the peripheral nervous system with an annual incidence of approximately 0.6-4.0 per 100,000 people (1). Ascending paralysis, defined as weakness beginning in the feet and hands and migrating towards the trunk, or descending paralysis, defined as weakness moving from the proximal regions to the distal regions, are the most common symptoms of GBS. In most patients, the disease course is self-limited; the symptoms peak within two weeks and recover within several weeks to months. However, the mortality rate secondary to acute paralysis of the respiratory muscles remains 1.7%-5%. Timely and effective treatment in the acute phase is therefore crucial in clinical practice (2).

Both cellular and humoral immunity are generally believed to be involved in the pathogenesis of GBS, and various types of activated $CD4^+$ T cells orchestrate the onset and progression of GBS (3). $CD4^+$ T cells are capable of attracting and activating other immune cells, including CD8+ T lymphocytes, B lymphocytes, neutrophils and macrophages. Based on their immunological functions, $CD4^+$ T cells are categorized as Th1, Th2, Th17 and Treg cells. Under physiological conditions, the levels of Th1 and Th2 cells are in a dynamic balance. Multiple studies have demonstrated that the Th1/Th2 ratio in peripheral blood is increased in GBS patients (4). Cytokines secreted by Th1 cells such as IFN-γ and IL-2, play an important role in the breakdown of the blood-brain barrier, the blood-nerve barrier and the loss of the myelin sheath. GBS patients tend to have a poor prognosis if their levels of soluble IFN receptors or IL-2 receptors are significantly increased in the peripheral blood at the acute stage (5), suggesting that Th1 cells may be the main effector cells that mediate peripheral nerve injury in GBS patients. Studies have demonstrated that increasing numbers Th2 cells are related to recovery in experimental autoimmune neuritis (EAN), a GBS animal model, suggesting that increased Th2 cells are a protective factor of the immune response in EAN (6). The relationship between Th17 cells and autoimmune diseases has been under extensive investigation. Studies have confirmed that Th17 cells play an important role in the pathogenesis of many conditions, including multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis (7-9). Treg cells which expressing the transcrip-

tion factor forkhead box protein 3 (FoxP3) are a specific subtype of immune suppressive cells that suppress the activation, proliferation and effector functions of various effector immune cells, especially inhibit effector T cell proliferation and therefore restrain the development of excessive immune responses (10,11). Reduced levels or an impaired function of Tregs have been described in human patients with GBS (12-14) and CIDP (15,16). In corresponding animal models, previous studies have demonstrated that in GBS patients, the Th17/Treg ratio is imbalanced, such that Th17 cells are hyperactive, whereas Treg cells are decreased. One study on Th17 cells and EAN (17) reported that the level of Th17 cells is elevated initially and decreases as symptoms improve, suggesting that Th17 cells are involved in the pathogenesis of GBS. Pritchard et al. (18) demonstrated that in GBS, Treg cells are significantly reduced, leading to loss of inhibition of $CD4^+$ T cells and potentially promoting disease development. Research on the function of Th17 and Treg cells in GBS has been limited, and most studies are based on animal models.

Plasma exchange (PE), is used in the treatment of GBS for years (19). In the past years, there are several papers regarding the application of PE in the therapy of neuroimmunological disease as well as in other autoimmune disease. However, the application of PE in GBS has rarely been reported (20-22), moreover, the mechanism of the therapeutic effect seldom been investigated. Lymphoplasma exchange (LPE) is a relatively new blood transfusion technology which was developed from PE recently. Compare to regular PE, LPE not only removes pathological substances from patient plasma but also selectively eliminates the immuno-competent cells of PBMC, especially lymphocytes which are the main source of these pathological molecules (23). Therefore, it is taken for that the effects of LPE are relatively long-lasting relative to PE. At present, there is no English literature on the treatment of GBS by the LPE, let alone the mechanism of LPE in the treatment of GBS.

The clinical evaluation of GBS relies on two scoring systems: the Hughes score (2,3) and The British Medical Research Council (MRC) score. The Hughes score is based on the evaluation of walking and physical exertion capacities, the requirement for mechanical ventilation and other measures. While the MRC score, a scale to grade muscle strength from 0 to 5 developed by The British Medical Research Council, focuses on specific assessments, including bilateral shoulder abduction, forearm flexion, wrist extension, thigh flexion, knee extension, and foot dorsiflexion force. Since the MRC assigns the strength scores of 6 muscle groups bilaterally, it is a more exact reflection of the neurological deficit of the patient and is likely more sensitive than the Hughes score in this regard.

The present study compared the MRC and Hughes scores before and after LPE treatment of GBS. Furthermore, we closely monitored the serum immune-related indicators of GBS patients in groups, quantitatively evaluated the efficacy of the LPE treatment in GBS, and investigated its therapeutic mechanism.

Materials and Methods

Patients and Specimens

Twenty-four patients who were diagnosed with GBS based on the GBS Van der Meche FG 2001 and Wakerley BR 2014 (24,25). Revised criteria underwent LPE at the Department of Neurology at Xiangya Hospital of Central South University between 2014 and 2016. There were 13 men and 11 women, with an average age of 38.5 ± 5.74 years. The exclusion criteria were as follows: peak time longer than 2 weeks; associated with other autoimmune diseases; the administration of pulse steroid therapy, high-dose intravenous immunoglobulin, or plasma exchange therapy; and had contraindications of lymph plasma exchange treatment. The average treatment time was 7.5±1.8 days. Plasma samples were also obtained from 12 healthy age and sex-matched control subjects (NCs) (7 men, 5 women, 40.5±4.35 years old). Written informed consent was obtained from all subjects.

Treatment protocol

LPE treatment using COBE Spectra blood cell separator, installed a white cell channel, the collection bag replaced for the 3000ml big bag, using density gradient centrifugation and photoelectricity technology, entering the manual program when the lymphoid cells collected, clipping the feedback channel of patients'autologous plasma to make it into the collection bag along with peripheral blood mononuclear cell (PBMC) lymphocytes is removed, while injectiing the fresh frozen plasma from the clamp at the top left of heparin cap, which reinfused to patient's body after confluence of the patient's red blood cells.

When patients received LPE therapy, their autoantibodies, alloantibodies, circulating immune complexes, inflammatory mediators and other pathological substances in plasma were removed, at the same time, immunocompetent cells of PBMC which closely related with various autoimmune diseases (AID) can also be selectively removed, especially lymphocytes. Even all the blood cells harmful to the body may be removed together such as pathological plasma cells, antigen presenting cells (APC), leukemia cells, tumor cells, allogeneic lymphocytes of GVHD patients, etc. To adjust the number and concentration of the remaining lymphocytes at any time, the separator monitored the number and concentration of the removed lymphocytes throughout the process of replacement.

Vital signs were monitored at the beginning and end of each procedure, and patients were monitored for adverse events during the apheresis procedures. Written informed consent was obtained from all patients after procedural risks were explained in detail before each procedure. Possible adverse reactions to LPE, such as allergic reactions, hypotension, and hypocalcemia, were closely monitored. Oral calcium gluconate, non-Flanagan intramuscular injection, and intravenous injection of dexamethasone were administered when necessary. Electrocardiogram monitoring was performed in patients with concurrent cardiovascular diseases.

Indicators

The neurological deficit scores for all patients were

scored pre-treatment and post-treatment (2 days, 4 days, 1 week, and 2 weeks). The Hughes scores (2,3) were recorded as follows: 0, normal, no neurological deficit; 1, mild signs and symptoms, general physical labor; 2, able to walk without help; 3, able to walk with help; 4, requires the use of a wheelchair or bed; 5, requires mechanical ventilation; and 6, death. The MRC sum score (0 to 5 scale) is based on the strength of six muscles bilaterally as follows: shoulder abduction, forearm flexion, wrist stretch, thigh flexion, knee extension, and foot dorsiflexion. The total muscle score ranged from 0 (complete paralysis) to 60 (normal). The efficacy evaluation standards were as follows: marked, Hughes score returned to 0 or 1 within 2 weeks after treatment, with the disappearance of cranial nerve palsy and other clinical signs; improved, Hughes score improved 1 or 2 points, or the MRC score of at least two limbs improved at least 1 point within 2 weeks after treatment; ineffective, the Hughes score and MRC score did not change or declined, and no improvement in the bulbar paralysis or the paralysis of respiratory muscles was observed. Marked and improved scores were classified as effective.

Biochemical indicators: the levels of peripheral blood immunoglobulins (IgG, IgA and IgM), complement (C3, C4), peripheral blood mononuclear cells (PBMCs), fibrinogen, and lymphocyte subsets were evaluated prior to treatment and two days after the treatment. Peripheral blood mononuclear cells (PBMCs) were tested using a PC-based 5-part Auto Hematology Analyzer (CLS-8800, China). The levels of peripheral blood immunoglobulins and complements were tested using an automatic biochemical-immune analyzer (MY-B020, China). Fibrinogen was measured using a fully automatic blood coagulation analyzer (STAGO, France), and T lymphocyte subsets were quantified using flow cytometry (BD, USA).

Flow cytometry

Five milliliters of peripheral blood samples from 12 GBS patients were obtained prior to the start of LPE treatment and 2 days, 4 days, 1 week and 2 weeks after LPE treatment, 12 healthy controls (age and sex-matched) . PBMCs were isolated by Ficoll density gradient centrifugation, and the cell concentration was adjusted to 2×10^6/ml. Mononuclear cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml; Sigma, St. Louis, MO, USA) and ionomycin (1 μg/ml; Sigma) in the presence of Brefeldin A (10 μg/ml; Sigma) for 4 h. For $CD4^+$ T lymphocyte subset analysis, Th1, Th2, and Th17 cells were first incubated with FITC-labeled anti-CD4 human antibodies for 20 min, fixed and permeabilized with a Perm/Fix solution, and using the following mouse anti-human monoclonal antibodies (mAbs) and corresponding isotype control antibodies (Abs) analyzed by flow cytometry: phycoerythrin- (PE-) conjugated IL-4, phycoerythrin- (PE-) conjugated IL-17A and Alexa Fluor 647-conjugated IFN-γ and appropriate isotype controls of mouse immunoglobulin G1 (IgG1). For Treg analysis, cells were incubated with Flourescein isothiocyanate isomer 1- (FITC-) conjugated anti-human CD4 antibodies and phycoerythrin- (PE-) conjugated anti-human CD25 for 20 min at 4 °C in the dark. After surface staining, the cells were permeabilized, fixed, and stained with Alexa Flour 647 anti-human Foxp3 according to the manufacturer's instructions. Isotype controls were used to correct compensation and confirm antibody specificity. All antibodies were purchased from BD Bioscience, NJ, USA. Flow cytometric analysis was performed on a FACSCalibur cytometer. Lymphocytes were gated according to their forward and size scatter characteristic. Fluorescence signals were collected in log mode. Ten thousand lymphocytes were acquired for each sample. Data processing was accomplished with CELLQuest software (BD).

Statistical analysis

The measurement data were analyzed using a variance test, and comparison between two groups was achieved using the independent sample t-test and correlation analysis. Data processing and statistical analyses were completed using SPSS for Windows (version 22.0). P-values <0.05 were considered statistically significant.

Results

Changes in neurological deficit score

The MRC scores and Hughes scores of each patient were significantly changed before and after treatment (Table 1).

Changes in immunoglobulin and complement levels and the percentage of monocytes and fibrinogen

The difference in immunoglobulin (IgG, IgA and IgM) and complement (C3, C4) levels between pre-treatment and post-treatment was statistically significant (all P<0.05, Table 2). The percentage of mononuclear cells and fibrinogen after LPE revealed differences relative to the levels before treatment and are statistically significant (all P<0.05, Table 3).

Changes in white blood cell counts in peripheral blood of patients with LPE treatment

In order to clarify whether the LPE can effectively remove lymphocytes, GBS patients' peripheral blood before and after treatment and the replacement fluid were taken Five classification blood cell detection. We focus on the number of white blood cells, lymphocytes and monocytes, compared to the previous treatment seen

Table 1. Changes in neurological deficit scores ($\bar{x}$ ±s).

Subject	n	Pre-treatment	Post-treatment			
			2 days	4 days	1 week	2 weeks
Hughes	24	3.7±0.76	3.6±0.69	3.6±0.81	3.1±0.73*	2.8±0.77*
MRC	24	25.7±10.4	32.4±10.2Δ	28.8±11.1Δ	36.7±10.4Δ	41.9±11.3Δ

The neurological deficit scores for all of the patients were scored pre- and post-treatment. *P=0.027 (1 week) and P=0.021 (2 weeks) versus before treatment. ΔP=0.025 (2 days), P=0.037 (4 days), P=0.019 (1 week), and P=0.013 (2 weeks) versus before treatment.

Table 2. Changes in immunoglobulin and complement levels ($\bar{x}$ ±s).

Subject	N	IgG	IgM	IgA	C3	C4
Pre-treatment	24	8.92±4.05	1.26±0.34	2.57±0.55	0.79±0.13	0.17±0.03
Post-treatment	24	7.38±3.62*	0.93±0.16*	2.03±0.51*	0.61±0.38*	0.13±0.02*
p value		0.017	0.032	0.028	<0.001	0.041

The immunoglobulin (IgG, IgA and IgM) and complement (C3, C4) levels in the patients' blood both pre- and post-treatment were tested by our hospital laboratory. *P<0.05 versus before treatment.

Table 3. Changes in monocyte and fibrinogen levels ($\bar{x}$ ±s).

Subject	Pre-treatment	Post-treatment	p value
PBMC count (10^9/L)	3.31±0.55	2.53±0.61*	0.041
PBMC percentage (%)	39.94±6.83	30.57±5.26*	0.026
Mononuclear cell count (10^9/L)	0.65±0.17	0.42±0.16*	0.033
Mononuclear cell percentage (%)	8.72±0.54	5.34±0.71*	0.036
Fibrinogen (g/L)	3.96±1.01	2.42±0.75*	0.029

Dynamic monitoring of the patients' monocyte and fibrinogen levels before and after lymphoplasma exchange was undertaken by examining their blood and coagulation functions. *P<0.05 versus before treatment.

in peripheral blood lymphocytes and monocytes were significantly decreased, with statistical significance.

Changes in CD4+ T lymphocytes subsets

Because of experimental success rates and other reasons, finally, only 12 cases of GBS patients did the flow cytometry assy, therefore 12 controls were taken. Before LPE therapy, the frequency of Th1 cells in GBS patients was higher than in NC (29.23±7.56% vs. 16.84±5.73%, p=0.027). Two days after therapy, the frequency of Th1 cells (21.34±5.08%) was obviously reduced (vs.29.23±7.56%, p=0.031) but remained higher than in the NCs (vs.16.84±5.73%, p=0.033). Four days after therapy, the frequency (22.18±4.95%) was marginally increased relative to two days after treatment which was not statistically significant (vs.21.34±5.08%, p=0.087), although it is get statistically significant relative to NCs (p=0.036). The frequency of Th2 cells in GBS patients was lower than that of NCs (3.27±1.73% vs. 5.08±1.87%, p=0.031) and increased two days(3.94±2.16%) and four days (3.72±1.35%) after therapy, although the trend was not statistically significant compared to the level before LPE treatment(p=0.037).

The proportions of T-helper cells (CD4) were similar in GBS patients and in NCs (p=0.084). The Th1/Th2 ratio in GBS patients was reduced two days after therapy (5.68±1.35 vs. 9.24±3.67, p=0.023) and slightly increased four days after therapy (5.94±1.62, p=0.027). All Th1 and Th2 levels and Th1/Th2 ratios were nevertheless higher than those in NCs (p=0.039, p=0.042, Table 5).

In this study, PBMCs from GBS patients were analysed by staining of surface CD4 and intracellular T cell cytokines. We selected typical flow cytometry picture, visible in the control group, the frequency of Th1 cells and Th2 cells in CD4+ T cells were 16.51% and 5.14% (Fig1-a, Fig2-a), while in GBS patients, the frequency of Th1 cells increased to 29.28% (Fig1-b) and the frequency of Th2 cells decreased to 3.23% (Fig2-b). Two days after LPE treatment, the frequency of Th1 cells in the peripheral blood of the same patient decreased to 21.28% (Fig1-c), compared with before treatment are statistically significant. Retest in the fourth day after treatment, the frequency of Th1 cells recovered slightly (22.%, Fig1-d), but still decreased significantly compared with before treatment. However, the frequency of Th2 cells of GBS patient did not differ significantly before and after LPE treatment (Fig2-b,c,d).

Before LPE therapy, the frequency of Th17 cells in GBS patients was higher than in NCs (1.94±0.61%

Table 4. Changes in white blood cell counts in peripheral blood due to replacement.

Subject	Pre-treatment	Post-treatment	Replacement fluid
WBC count (10^9/L)	8.77±3.26	6.83±3.36	5.00±2.33
Lymphocyte count (10^9/L)	2.57±1.32	1.97±0.27*	3.68±1.32
Neutrophil count (10^9/L)	5.63±2.92	4.19±2.20	1.16±1.22
Mononuclear cell count (10^9/L)	0.65±0.17	0.59±0.08*	0.57±0.06

*t=3.7428 (lymphocyte post-treatment), 4.1735 (mononuclear cell post-treatment), P<0.05, versus before treatment.

Table 5. Changes in the frequency of Th1 and Th2 cells.

Subjects	Cases	CD4+ T (%)	Th1 (%)	Th2 (%)	Th1/Th2
Normal control (NC)	12	40.76±40.35	16.84±5.73	5.08±1.87	3.31±1.04
Pre-treatment	12	39.87±10.23	29.23±7.56$^{\Delta}$	3.27±1.73$^{\Delta}$	9.24±3.67$^{\Delta}$
Two days post-treatment	12	40.65±11.76	21.34±5.08$^{*\Delta}$	3.94±2.16$^{\Delta}$	5.68±1.35$^{*\Delta}$
Four days post-treatment	12	41.33±11.01	22.18±4.95$^{*\Delta}$	3.72±1.35$^{\Delta}$	5.94±1.62$^{*\Delta}$

The percentages of Th1 and Th2 cells in the CD4+ T lymphocyte subsets were tested by FCS. *, versus pre-treatment; $^{\Delta}$, versus NCs.

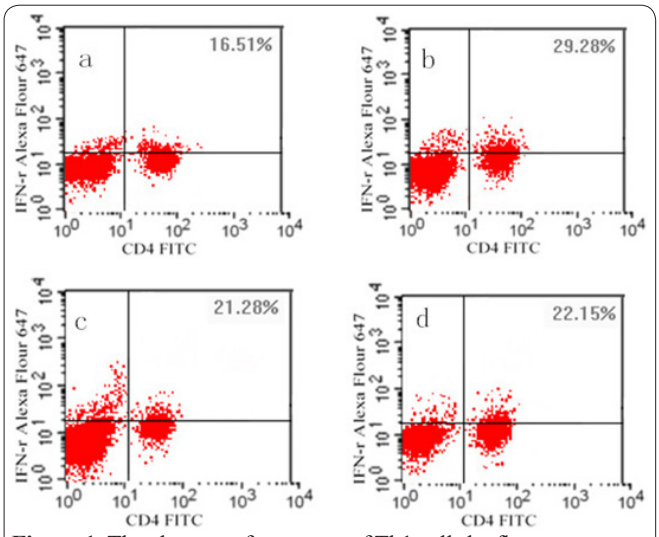

Figure 1. The changes of requency of Th1 cells by flow cytometry: strategy for the analysis of Th1 lymphocytes. Dot plots shown are representative of one healthy volunteer (a) and one patient with Guillain-Barré syndrome (b,c,d). Lymphocytes were first gated(denoted R1 in this figure) after heparinized peripheral whole blood from all subjects stimulated for 4 h ex vivo with phorbol myristate acetate (PMA) and ionomycin in the presence of monensin. Then cells were gated on $CD4^+$ lymphocytes(denoted R2 in this figure) and studied for expression of IFN-γ. a, one samples of normal control group; b, one day before treatment; c, 2 days after LPE treatment; and d, 4 days after LPE treatment. b,c,d were the same patients blood sample in the GBS group treated by LPE.

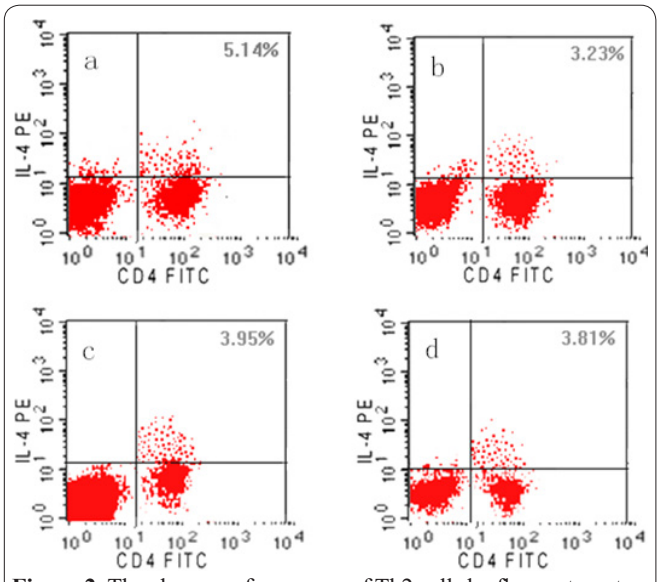

Figure 2. The changes of requency of Th2 cells by flow cytometry: strategy for the analysis of Th2 lymphocytes. Dot plots shown are representative of one healthy volunteer (a) and one patient with Guillain-Barré syndrome (b,c,d). Lymphocytes were first gated(denoted R1 in this figure) after heparinized peripheral whole blood from all subjects stimulated for 4 h ex vivo with phorbol myristate acetate (PMA) and ionomycin in the presence of monensin. Then after gating on $CD4^+$ cells(denoted R2 in this figure) , we detected the percentages of Th2 cells($CD4^+$ $IL4^+$) . a, one samples of normal control group; b, one day before treatment; c, 2 days after LPE treatment; and d, 4 days after LPE treatment. b,c,d were the same patients blood sample in the GBS group treated by LPE.

vs. 0.91±0.37%, p=0.032). Two days after therapy, the frequency of Th17 cells (1.13±0.28%) was lower than which of before treatment groug but higher than NCs group (P=0.037). Four days after therapy, the frequency of Th17 cells (1.25±0.49%) was recovered, but the difference did not reach statistical significance (P=0.068). The frequency of Treg cells in GBS patients was lower than in NCs (2.27±0.78% vs. 4.31±0.93%), and the difference was statistically significant (P=0.028). Two days after therapy, the frequency of Treg cells (3.34±0.65%) was lower than in NCs but higher than the levels prior to treatment; the differences were statistically significant (P=0.036). Four days after therapy, the frequency of Treg cells (3.02±0.62%) was higher than two days after treatment, but the difference was not statistically significant (P=0.083).

The Th17/Treg ratio in GBS patients was higher than in NCs (0.89±0.37 vs. 0.21±0.06, P=0.026). Two days after therapy, the Th17/Treg ratio (0.38±0.12) was lower than prior to treatment but higher than in NCs (P=0.033). Four days after therapy, the Th17/Treg ratio (0.43±0.15) was slightly higher than the level two days after treatment (P=0.071) (Table 6, Fig. 3&4).

The frequency of Th17 cells and Treg cells in CD4+ T cells of healthy people were 0.73% and 4.51% (Fig3-a, Fig4-a), while in GBS patients, the frequency of Th17 cells increased to 2.24% (Fig3-b) and the frequency of Treg cells decreased to 2.14% (Fig4-b).2 days after LPE treatment, the frequency of Th17 cells in the peripheral blood of the same patient decreased to 1.12% (Fig3-c), and the frequency of Tregs increased to 3.43% (Fig4-c), compared with before treatment are statistically significant. Retest in the fourth day after treatment, the frequency of Th17 cells recovered slightly (1.25.%, Fig3-d), but still decreased significantly compared with before treatment. Meanwhile, the frequency of Treg cells also recovered slightly (3.05.%, Fig4-d), compared with before treatment are statistically significant.

Correlation analysis

Before LPE therapy, the numbers of Th1 and Th17 cells and the Th1/Th2 and Th17/Treg ratios in GBS patients were inversely correlated with MRC scores (r=-0.745, P<0.001; r=-0.692, P=0.004; r=-0.802, P<0.001; and r=-0.764, P<0.001, respectively), whereas those of Th2 and Treg cells were positively correlated (r=0.561, P=0.042 and r=0.758, P=0.013, respectively).

Table 6. Changes in the frequency of Th17 and Treg cells.

Subjects	Cases	Th17 (%)	Treg (%)	Th17/Treg
Normal control (NC)	12	0.91±0.37	4.31±0.93	0.21±0.06
Pre-treatment	12	1.94±0.61$^{\Delta}$	2.27±0.78$^{\Delta}$	0.89±0.37$^{\Delta}$
Two days post-treatment	12	1.13±0.28*	3.34±0.65*$^{\Delta}$	0.38±0.12*
Four days post-treatment	12	1.25±0.49*	3.02±0.62*$^{\Delta}$	0.43±0.15*

The percentages of Th17 and Treg cells in the CD4+ T lymphocyte subsets were tested by FCS. *, versus before treatment; Δ, versus NCs.

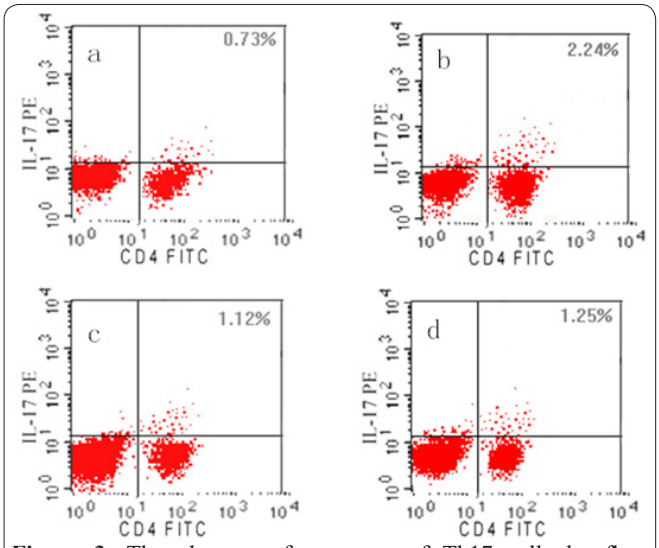

Figure 3. The changes of requency of Th17 cells by flow cytometry: strategy for the analysis of Th17 lymphocytes. Dot plots shown are representative of one healthy volunteer (a) and one patient with Guillain-Barré syndrome (b,c,d). Lymphocytes were first gated(denoted R1 in this figure) after heparinized peripheral whole blood from all subjects stimulated for 4 h ex vivo with phorbol myristate acetate (PMA) and ionomycin in the presence of monensin. Then cells were gated on $CD4^+$ lymphocytes(denoted R2 in this figure) and studied for expression of IL-17. a, one samples of normal control group; b, one day before treatment; c, 2 days after LPE treatment; and d, 4 days after LPE treatment. b,c,d were the same patients blood sample in the GBS group treated by LPE.

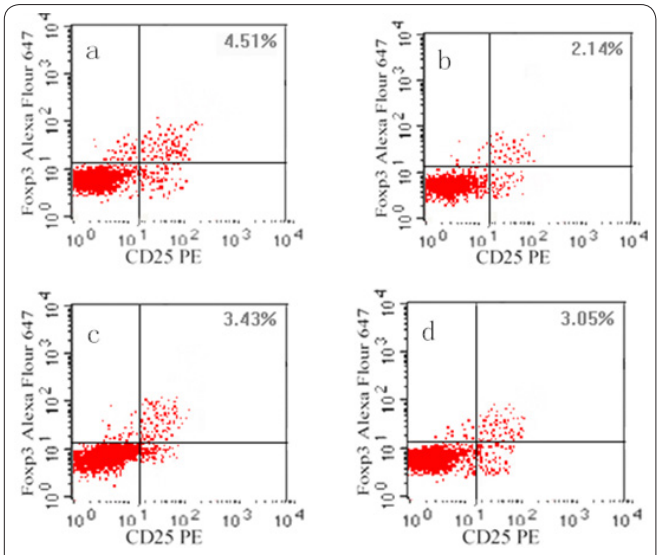

Figure 4. The changes of requency of Treg cells by flow cytometry: After gating on $CD4^+$ cells(denoted R2 in this figure), we detected the percentages of Treg cells ($CD4^+$ $CD25^+$ $FoxP3^+$) . a, one samples of normal control group; b, one day before treatment; c, 2 days after LPE treatment; and d, 4 days after LPE treatment. b,c,d were the same patients blood sample in the GBS group treated by LPE.

Two days after LPE therapy, the frequency of Th1, Th2, Th17, and Treg cells and the levels of immunoglobulin, complement, fibrinogen, PBMCs and mononuclear cells in GBS patients were no longer correlated with the MRC scores. The improvement in MRC scores in GBS patients was correlated with the decrease in the numbers of Th1, Th17, and Tregs as well as in the Th1/Th2 and Th17/Treg ratios ($r=0.653$, $p=0.043$; $r=0.721$, $p=0.034$; $r=0.786$, $p=0.037$; $r=0.682$, $p=0.041$; and $r=0.796$, $p=0.038$, respectively). Additionally, the improvement of the MRC scores in GBS patients was correlated with the increase in the number of Treg cells ($r=0.786$, $p=0.037$). The increase in Th2 cells was not significantly correlated with the improvement of the MRC scores.

Discussion

The principal pathological changes of GBS include segmental demyelination and macrophage infiltration of peripheral nerves. GBS is divided into several subtypes based on electrophysiological and pathological findings: acute inflammatory demyelinating polyneuropathies (AIDP), acute motor axon neuropathy (AMAN), acute motor-sensory axon neuropathy (AMSAN), Miller Fisher syndrome (MFS), acute pan-autonomous neuropathy and acute sensory neuropathy.

The pathogenesis of GBS is not entirely clear. It is generally accepted that both cellular and humoral immunity are involved in GBS, although to varying extents in different subtypes. For example, AIDP is primarily mediated by cellular immunity. T cells activated by currently unidentified pathogenic antigens penetrate the blood-nerve barrier and release pro-inflammatory cytokines, including TNF-α, IFN-γ and IL-2, which activate macrophages to release Nitric Oxide (NO), matrix metalloproteinases (MMPs), and other toxic molecules to directly damage myelin sheath. The differentiation of B cells leads to the secretion of antibodies and the activation of complement, which can cause demyelination and axonal degeneration (22). However, dysregulated humoral immunity predominates in AMAN and AMSAN. Anti-ganglioside antibodies (such as anti-GM, GM1a, GM1b and Ga1Nac-GD1a) activate complement, which attract macrophages to the fiber myelin-axonal gap, leading to axonal damage and degeneration. In approximately 80%-90% of MFS patients, the serum anti-GQ1b titer is highly elevated (26). The reason for the effectiveness of plasma exchange in treatment for GBS may lay in the rapid elimination of pathological substances, such as autoantibodies, alloantibodies, complement components, circulating immune complexes, a variety of cytokines, and endogenous and exogenous toxicants in patient serum. However, traditional plasma exchange does not remove activated inflammatory cells, such as sensitized T and B lymphocytes and other immunocompetent cells that produce pathological substances. With the decline of antibody titers in the blood circulation after plasma exchange, the removal of the feedback inhibition of lymphocytes causes a 'rebound' production of pathological materials within a short time. For example, the activation and proliferation of B cells may lead to rapid synthesis of antibody. The increased antibody production results in disease relapses (27). Therefore, repeated multiple plasma exchanges are essential to prevent antibody rebound. However, according to the study by Yuki et al. (28), although the first and second plasma exchanges can result in a 40-60% decrease of immunoglobulin levels, there is no significant decrease after the third exchange (22). In GBS, the optimal number of plasma exchange treatments has been addressed in one large multicenter study (21). In this controlled

non-blinded trial, 556 patients were randomized to three groups according to their disability. Mildly affected patients were treated with zero or two plasma exchange sessions, whereas moderately affected patients with two or four affected muscles and severely affected (mechanically ventilated) GBS patients received four or six plasma exchange treatments. Two versus zero treatments in the mildly affected patients and four versus two plasma exchange sessions in the moderately affected patients were more beneficial. More than four plasma exchange sessions did not yield additional benefit in severely affected patients. Yuki and colleagues (28) studied the reduction of serum immunoglobulin levels and serum anti-ganglioside antibody titers in GBS patients who underwent plasma exchange treatment. Consistent with the results from the aforementioned trial, serum immunoglobulin fractions decreased approximately 40-60% after two sessions but did not decline substantially in subsequent plasma exchange sessions.

LPE combines traditional plasma exchange with a lymphocyte elimination technique, cannot only remove the pathological materials in the blood but also eliminates activated T and B cells by removing PBMC, consequently preventing the production of antibodies and inflammatory cytokines. As a result, LPE is able to significantly enhance the efficacy of plasma exchange and prevent relapses (28,29). To further explore the mechanism of LPE, we monitored the changes in immunoglobulin and complement levels before and after treatment. Our results demonstrate that LPE reduced IgG, IgA and IgM levels significantly. A similar trend was also demonstrated for the levels of C3 and C4. These data suggest that the mechanism of LPE may be associated with the removal of pathological substances like immunoglobulin and complements cells as well as some immune active cells in the peripheral blood of GBS patients.

Extra-cellular matrix proteins, such as fiber-binding protein and laminin, play an important role in the repair of peripheral nerve injury. After a peripheral nerve is injured, excessive fibrinogen leaks from blood vessels and transforms into fibrin, thereby changing the composition of the extra-cellular matrix and affecting myelin synthesis. A previous clinical investigation demonstrated that a decline of ≥30% in fibrinogen concentration had a significant relationship with lasting neurological function improvement (30). In this study, fibrinogen levels in the peripheral blood significantly decreased after LPE therapy (see in Tab.3), indicating that the neurological function improvement of GBS patients is partly related to the removal of plasma fibrinogen and effective promotion of myelin regeneration.

Macrophages play an important role in the axonal injury and peripheral nerve demyelination of GBS patients. Previous studies (31) have demonstrated that the presence of circulating mononuclear cells invading myelin is an indispensable factor in GBS pathogenesis. Macrophages can activate T and B cells to induce immune responses through their efficient antigen-presenting capacity, which indirectly leads to peripheral nerve injury. Recently, Hu et al. (32) confirmed that IL-23 plays a key role in the effect phase of immune-mediated demyelination of peripheral nerves, and both macrophages and T helper-1 cells are the source of IL-23 cells. Our study found that the percentage of mononuclear cells in the blood was significantly decreased after LPE (Tab.3 and 4). Because lymphocytes and macrophages are the major constituents of mononuclear cells, the decreasing percentage of mononuclear cells indicates that LPE treatment of GBS may be related to the elimination of monocytes in the blood which reduce the damage to the peripheral nerves by macrophages and activated T and B lymphocytes.

Previous studies demonstrated different outcomes with the detection of GBS patients' lymphocyte subsets. A variety of immune cell subsets and a complex network of cytokines are involved in the pathogenesis of GBS and EAN. Th1, Th2, Th17 and Treg cells, the four common subsets of $CD4^+$ T cells, antagonize or restrict each other by releasing their effector cytokines. The unbalance of Th1 and Th2 cells has a direct relevance to autoimmune responses, which has been observed in several autoimmune diseases, such as GBS and multiple sclerosis (MS), an autoimmune inflammatory demyelinating disease affecting the central nervous systems (CNS) in humans. Our study found that before treatment, the clinical symptom severity in GBS patients was positively correlated with Th1 cell frequency and was negatively correlated with the frequency of Th2 cells, which is consistent with previous research (33,34). Two days after LPE treatment, GBS patients exhibited a decreased frequency of Th1 cells (Table 5& Figure 1), and clinical score improvement was correlated with the decreased frequency of Th1 cells (Table 7&8). LPE is able to quickly and effectively remove pathogenic antibodies, complement components, and other inflammatory factors in patients. In the present study, after LPE treatment, $CD4^+$ T cells were polarized to Th2 instead of Th1 differentiation. Thus, the Th1/Th2 cell ratio was decreased and favored immune inhibition. The changes in $CD4^+$ T lymphocyte subsets could be explained as the decreased levels of pro-inflammatory cytokines by LPE. Four days after treatment, Th1 cells in the peripheral blood of patients were mildly increased relative to measurements after 2 days (Table 5& Figure 1), implying that a new round of inflammatory cell activation

Table 7. Correlation analysis between CD4+ T cell numbers before LPE and clinical scores.

Subject	Cases	Frequency	Correlation coefficient (r)	p value
Th1	12	29.23±7.56	-0.745*	<0.001
Th2	12	3.27±1.73	0.561	0.042
Th17	12	1.94±0.61	-0.692*	0.004
Treg	12	2.27±0.78	0.758*	0.013
Th1/Th2	12	9.24±3.67	-0.802*	<0.001
Th17/Treg	12	0.89±0.37	-0.764*	<0.001

The changes in the CD4+T lymphocyte subsets were also correlated with changes in the patient MRC scores. * P<0.05.

Table 8. Correlation analysis between the frequencies of Th cells and the immunoglobulin, complement, fibrinogen, PBMC and mononuclear cell levels in GBS patients

Subject	cases	Change of frequency	Correlation coefficient (r)	p value
Th1	12	7.64±3.03	0.653*	0.043
Th2	12	0.62±0.25	0.462	0.097
Th17	12	0.71±0.34	0.721*	0.034
Treg	12	1.02±0.46	0.786*	0.037
Th1/Th2	12	3.73±1.02	0.682*	0.041
Th17/Treg	12	0.46±0.17	0.796*	0.038

* $P<0.05$.

may be generated; the mechanism and significance of this process are discussed below.

Similar to Th1 cells, the production of Th17 cells is suppressed after LPE. We speculate that due to the reduction of inflammatory cytokines and the antigen stimulation after LPE treatment, the immune balance of GBS patients tends to be normalized or even suppressed in the rebuilding process, which lead to decreased production of the suppressor/inducer T cell subsets that could provide help for the secretion of circulating autoantibodies directed to peripheral myelin components. The present study demonstrated that the frequency of Th17 cells in the acute phase of GBS patients was significantly higher than that of the NC group. The frequency of Th17 cells was also related to disease severity, which is consistent with the results of animal studies. Previous studies on animal models of GBS suggest that Th17 cells may secrete IL-17, which promotes the production of chemokines, adhesion molecules, and colony-stimulating factor to promote an inflammatory response. In our study, the frequency of Th17 cells in the peripheral blood of GBS patients was decreased two days after LPE treatment, the degree of the decrease was positively correlated with the improvement in clinical scores (Table 7&8), suggesting that LPE therapy may improve symptoms by reducing Th17 cells in patients' peripheral blood.

$CD4^+$ $CD25^+$ Tregs is a group of T cell subsets with a unique feature, which participated in the active suppression process of potentially autoreactive T cells (35), inhibiting the proliferation of $CD4^+$ T cells primarily through direct contact between cells (36). Animal experiments confirmed that susceptible mice would be induced to occurs $CD4^+$ T cell-mediated autoimmune diseases by removed $CD4^+CD25^+$ Tregs in the peripheral blood, and Treg reinfusion in vivo is able to inhibit the onset of sensitized mice (37). This shows the change in the number of $CD4^+CD25^+$ Tregs plays an important role in the pathogenesis of autoimmune diseases. GBS is a $CD4^+$ T cell-dependent autoimmune disease, $CD4^+$ T cell activation is the key to the disease occurred (38). Presumably, GBS patients may exist the imbalance of proportion of Treg cells, leading to loss of inhibition of $CD4^+$ T cell. Very little is known about the relation between Tregs and GBS. The only two studies (14,39) on the frequency of $CD4^+CD25^+$T cells in GBS patients are controversial. One study found that the number and proportion of $CD4^+CD25^+$ T cells were reduced in acute GBS compared with controls. However, another found no difference between GBS and controls in the percentage of circulating CD4+CD25+ T lymphocytes. Our study found that, in contrast to the NC group, the frequency of Treg cells in GBS patients in the acute phase of the disease was significantly reduced and was negatively correlated with clinical severity. Moreover, the frequency of Treg cells increased, and the improvement of clinical symptoms were positively correlated (Table 7&8).

Studies have confirmed that Treg cells and Th17 cells exhibit mutual inhibitory effects. $CD4^+$ T cells in the presence of TGF-β alone are induced to differentiate into immunosuppressive Treg cells. In the presence of TGF-β and IL-6, $CD4^+$ T cells differentiate into Th17 cells (18). Foxp3 is a specific transcription factor involved in Treg cell regulation and inhibition of RORγt function, which reduces the differentiation of Th17 cells. In T cells expressing both Foxp3 and RORγt, the RORγt inhibition of Foxp3 can be eliminated by IL-6 or IL-21, which increases the differentiation of Th17 cells (40). Studies have confirmed that IL-35 secreted primarily by Treg cells is a negative regulator of immune effector molecules that can inhibit the in vivo differentiation of Th17 cells and IL-17 production (41); the multiple reductions of pro-inflammatory cytokines can promote increased Foxp3 and reduced RORγt levels, ultimately promoting the differentiation of Treg cells. The imbalance between Th17 and Treg cells in the pathogenesis of GBS is the focus of the current study. The results of our study demonstrate that the Th17/Treg cell ratio was significantly higher in GBS patients than in the NC group and was positively correlated with clinical severity (Table 8). This finding suggests that the incidence of GBS may not simply result in increased Th17 cells or decreased Treg cells but that the interactions between Th17 cell and Treg cell leading to immune imbalance. It may be possible for LPE to restore the balance of the Th17/Treg cell ratio in GBS patients for therapeutic purposes.

Previous study about the pathological of Guillain-Barre syndrome show: (1) the incidence of nerve fibers in the first 3 to 4 days began to resemble edema; (2) 4 to 5 days later, demyelination with axonal mild swelling was observed; and (3) 8 days after the first observation of cell infiltration, i.e., on day 12, macrophage infiltration and nerve sheath cell proliferation was observed. These findings suggest that the peripheral neuropathic damage occurred mainly during the initial 4 to 8 days. Based on the course of the disease, Guillain-Barre syndrome is a self-limiting disease. The duration of the peak is generally approximately 2 weeks. After the peak is reached, the complement in the serum of patients, which combines with anti-peripheral nerve myelin antibodies, presented a rapid natural decrease. Therefore, the lymph plasma exchange treatment should be conducted as ear-

ly as possible, particularly the first week after disease onset, because the effect of this treatment is not obvious after the peak period. To eliminate the effects of the natural course of immunological indexes in patients, this study included only the patients that underwent lymph plasma exchange within two weeks of onset and therefore cannot compare the efficacy of different timings of the lymph plasma exchange treatment. To determine the appropriate times of LPE treatments for GBS patients, we recorded the neurological deficit scores and the changes in related immunology indicators of GBS patients at different times after LPE treatment. We found that relative to pre-treatment levels, the levels of mononuclear cells, immunoglobulins, complement, and fibrinogen were decreased significantly 2 days after LPE treatment, the imbalance of $CD4^+$ T lymphocyte subsets was partially corrected, and the neurological deficit scores were also improved significantly. The clinical symptoms also exhibited a marked improvement 4 days after treatment. However, compared with 2 days post-treatment, on 4 days post-treatment, the Th1 and Th17 cell frequencies were slightly higher, the Treg cell frequency was decreased, and the MRC scores were also aggravated, which indicates the presence of a new generation of pathogenic cells. It is possible that one LPE treatment cannot completely remove the hazardous substances such that the auto-antigen continues to stimulate the initial CD4+ T cells primarily differentiating to the effector Th1 or Th17 cells. Alternatively, it is possible that after LPE treatment, the residual antigens remain capable of promoting the differentiation of memory cells into effector cells. This finding indicates that one LPE treatment may not be able to consolidate the curative effect. We recommend that LPE treatment should be performed at least twice. The circle of lymphoblast proliferation and antibody production is inhibited by multiple LPE treatments, such that the patient's immune system is gradually restored to normal. Due to the small sample size of this study, the efficacy of different timings of LPE treatments could not be stratified. In addition, the observation time was too short to observe a decrease in the level of pathogenic lymphocytes after the peak level was reached following LPE treatment. As a result, we only showed that the frequency of Th1 and Th17 cells was increased in the 4 days after treatment. Besides, Schumak et al. demonstrated that multiple lymph plasma exchanges should be avoided within a short interval because the efficacy of these treatments would decrease. yuki et al. (28) also reported that the first two plasma exchanges can reduce the level of immunoglobulins by 40-60%, whereas the third exchange fails to reduce immunoglobulin levels. However, the "Guidelines on the Use of Therapeutic Apheresis in Clinical Practice" (19) suggest that five to six Therapeutic Apheresis (TPE) over 10-14 days are recommended. On the basis of the traditional plasma exchange, LPE focus on removing immunocompetent cells constituted mainly by lymphocytes. Moreover, in the clinical practice of the use of LPE to treat GBS patients, we found that the disease did not recur after the second LPE treatment, and the neurological function deficit score had improved. Therefore, we believe that the effect to therapy GBS of LPE is better than TPE. And we postulated that two consecutive LPE treatments over 3-5 days may normalize the immune systems of patients with GBS and achieve better clinical efficacy.

Based on the previous research and this study, we propose the following suggestions for the use of LPE treatment in GBS patients. Firstly, since the pathological damage of GBS occurs in the first 4-8 days, LPE should be performed as early as possible. Secondly, LPE should be performed at least twice over 3-5 days. Thirdly, a more favorable therapeutic effect of LPE might be achieved if the patients exhibit the following abnormalities: (1) particularly high percentage of monocytes in the peripheral blood; (2) abnormal immunoglobulin levels or complement titer, particularly high IgM levels; (3) significant differences in lymphocyte subsets; or d. high fibrinogen levels. Finally, clinicians should be alert to the possible complications: hives, hypoglycemia, hypocalcemia, thrombosis, hemorrhage, pulmonary edema and anaphylactic shock.

Our study demonstrates that LPE treatment has a significant effect on GBS. The therapeutic mechanism is related to the correction of lymphocyte subsets disorder in the blood and the direct removal of pathological substances, such as immunoglobulin, complement, monocytes and fibrinogen. Due to the marked reduction in the number of times the patients have to undergo the treatments, the LPE treatment of GBS patients not only exhibits satisfactory clinical efficacy but also shortens the length of the patients' hospital stay, reduces the patients' pain and economic burden, and reduces the patients' risk of infection due to an invasive operation and the repeated transfusion of allogeneic blood. Further studies are required to investigate the efficacy of LPE and to explore its therapeutic mechanisms.

Disclosure of conflict of interest

The authors have declared that no competing interests exist.

Acknowledgments

Xu LQ, Zhang Z, Luo ZH and the Hematology Department Laboratory are gratefully acknowledged for their expert technical assistance.

Role of the Funding Source

This work was supported by the Hunan Provincial Innovation Foundation For Postgraduate under Grant number CX2013B106 of China.

References

1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. Neuroepidemiology. 2011;36:123-33.

2. Hughes RA, Cornblath DR. Guillain-Barré syndrome. Lancet. 2005;366:1653-66.

3. Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial prednisolone in acute polyneuropathy. Lancet. 1978;2:750-3.

4. Coffman RL. Origins of the T(H)1-T(H)2 model: a personal perspective. Nat Immunol. 2006;7:539-41.

5. Nyati KK, Prasad KN, Rizwan A, Verma A, Paliwal VK. TH1 and TH2 response to Campylobacter jejuni antigen in Guillain-Barre syndrome. Arch Neurol. 2011;68:445-52.

6. Hadden RD, Karch H, Hartung HP, Zielasek J, Weissbrich B,

Schubert J, Weishaupt A, Cornblath DR, Swan AV, Hughes RA, Toyka KV; Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. Neurology. 2001;56:758-65.
7. Zou LP, Abbas N, Volkmann I, Nennesmo I, Levi M, Wahren B, Winblad B, Hedlund G, Zhu J. Suppression of experimental autoimmune neuritis by ABR-215062 is associated with altered Th1/Th2 balance and inhibited migration of inflammatory cells into the peripheral nerve tissue. Neuropharmacology. 2002;42:731-9.
8. Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in Th cells. J Immunol. 2000;165:6107-15.
9. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, Skapenko A. Role of Th17 cells in human autoimmune arthritis. Arthritis Rheum. 2010;62:2876-85.
10. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. Cell. 2010;140:845-58.
11. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010;10:490-500.
12. Pritchard J, Makowska A, Gregson NA, Hayday AC, Hughes RA. Reduced circulating CD4+CD25+ cell populations in Guillain-Barré syndrome. J Neuroimmunol. 2007;183:232-8.
13. Harness J, McCombe PA. Increased levels of activated T-cells and reduced levels of CD4/CD25+ cells in peripheral blood of Guillain-Barré syndrome patients compared to controls. J Clin Neurosci. 2008;15:1031-5.
14. Chi LJ, Wang HB, Zhang Y, Wang WZ. Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barré syndrome. J Neuroimmunol. 2007;192:206-14.
15. Chi LJ, Wang HB, Wang WZ. Impairment of circulating CD4+CD25+ regulatory T cells in patients with chronic inflammatory demyelinating polyradiculoneuropathy. J Peripher Nerv Syst. 2008;13:54-63.
16. Sanvito L, Makowska A, Mahdi-Rogers M, Hadden RD, Peakman M, Gregson N, Nemni R, Hughes RA. Humoral and cellular immune responses to myelin protein peptides in chronic inflammatory demyelinating polyradiculoneuropathy. J Neurol Neurosurg Psychiatry. 2009;80:333-8.
17. Jadidi-Niaragh F, Mirshafiey A. Th17 cell, the new player of neuroinflammatory process in multiple sclerosis. Scand J Immunol. 2011;74:1-13.
18. Pritchard J, Makowska A, Gregson NA, Hayday AC, Hughes RA. Reduced circulating CD4+CD25+ cell populations in Guillain-Barré syndrome. J Neuroimmunol. 2007;183:232-8.
19. Schwartz J, Winters JL, Padmanabhan A, Balogun RA, Delaney M, Linenberger ML, Szczepiorkowski ZM, Williams ME, Wu Y, Shaz BH. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: the sixth special issue. J Clin Apher. 2013;28:145-284.
20. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986;136:2348-57.
21. Thomas E. Elliott, Barbara A. Elliott, Ronald R. Regal, Colleen M. Renier, Byron J. Crouse, David E. Appropriate number of plasma exchanges in Guillain-Barré syndrome. The French Cooperative Group on Plasma Exchange in Guillain-Barré. Syndrome. Ann Neurol. 1997;41:298-306.
22. Lehmann HC, Hartung HP. Plasma exchange and intravenous immunoglobulins: mechanism of action in immune-mediated neuropathies. J Neuroimmunol. 2011;231:61-9.
23. Zhang Z Zhang ZY, Fauser U, Schluesener HJ. Valproic acid attenuates inflammation in experimental autoimmune neuritis. Cell Mol Life Sci. 2008;65:4055-65.
24. Van der Meché FG, Van Doorn PA, Meulstee J, Jennekens FG; GBS-consensus group of the Dutch Neuromuscular Research Support Centre. Diagnostic and classification criteria for the Guillain-Barré syndrome. Eur Neurol. 2001;45:133-9.
25. Wakerley BR, Uncini A, Yuki N; GBS Classification Group; GBS Classification Group. Guillain-Barré and Miller Fisher syndromes--new diagnostic classification. Nat Rev Neurol. 2014;10:537-44.
26. Kieseier BC, Hartung HP, Wiendl H. Immune circuitry in the peripheral nervous system. Curr Opin Neurol. 2006;19:437-45.
27. Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. Brain. 2002;125:2591-625.
28. Yuki N, Tagawa Y, Hirata K. Minimal number of plasma exchanges needed to reduce immunoglobulin in Guillain-Barré syndrome. Neurology. 1998;51:875-7.
29. Berkman MZ, Zirh TA, Berkman K, Pamir MN. Tizanidine is an effective agent in the prevention of focal cerebral ischemia in rats: an experimental study. Surg Neurol. 1998;50:264-70; discussion 270-1.
30. Sanjay R, Flanagan J, Sodano D, Gorson KC, Ropper AH, Weinstein R. The acute phase reactant, fibrinogen, as a guide to plasma exchange therapy for acute Guillain-Barré syndrome. J Clin Apher. 2006;21:105-10.
31. Honma T, Sugimura K, Asakura H, Matsuzawa J, Suzuki K, Kobayashi M, Aoyagi Y. Leukocytapheresis is effective in inducing but not in maintaining remission in ulcerative colitis. J Clin Gastroenterol. 2005;39:886-90.
32. Hu W, Dehmel T, Pirhonen J, Hartung HP, Kieseier BC. Interleukin 23 in acute inflammatory demyelination of the peripheral nerve. Arch Neurol. 2006;63:858-64.
33. Hohnoki K, Inoue A, Koh CS. Elevated serum levels of IFN-gamma, IL-4 and TNF-alpha/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. J Neuroimmunol. 1998 1;87:27-32.
34. Li S, Jin T, Zhang HL, Yu H, Meng F, Concha Quezada H, Zhu J. Circulating Th17, Th22, and Th1 cells are elevated in the Guillain-Barré syndrome and downregulated by IVIg treatments. Mediators Inflamm. 2014;2014:740947.
35. Earle KE, Tang Q, Zhou X, Liu W, Zhu S, Bonyhadi ML, Bluestone JA. In vitro expanded human CD4+CD25+ regulatory T cells suppress effector T cell proliferation. Clin Immunol. 2005;115:3-9.
36. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med. 1998;188:287-96.
37. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, Masteller EL, McDevitt H, Bonyhadi M, Bluestone JA. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J Exp Med. 2004;199:1455-65.
38. Yoshii F, Shinohara Y. Lymphocyte subset proportions in Guillain-Barré syndrome patients treated with plasmapheresis. Eur Neurol. 2000;44:162-7.
39. Putnam AL, Vendrame F, Dotta F, Gottlieb PA. CD4+CD25high regulatory T cells in human autoimmune diabetes. J Autoimmun. 2005;24:55-62.
40. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol. 2007;8:345-50.
41. Kryczek I, Wei S, Zou L, Altuwaijri S, Szeliga W, Kolls J, Chang A, Zou W. Cutting edge: Th17 and regulatory T cell dynamics and the regulation by IL-2 in the tumor microenvironment. J Immunol. 2007;178:6730-3.