Clinical Immunology (2008) **126**, 180–188

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yclim

Decrease of $CD4^+CD25^{high}Foxp3^+$ regulatory T cells and elevation of $CD19^+BAFF\text{-}R^+$ B cells and soluble ICAM-1 in myasthenia gravis

Xiang Li [a], Bao-Guo Xiao [b], Jian-Ying Xi [a], Chuan-Zhen Lu [b], Jia-Hong Lu [a,*]

[a] *Department of Neurology, Hua Shan Hospital, Fudan University, Shanghai, 12 Middle Wulumuqi Road, 200040, China*
[b] *Institute of Neurology, Huashan Hospital, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China*

Received 27 June 2007; accepted with revision 3 October 2007

KEYWORDS
Myasthenia gravis;
Soluble factors;
Regulatory T cells;
B cells

Abstract Myasthenia gravis (MG) is caused by T-cell-dependent autoantibodies against muscle acetylcholine receptors (AChR) at the neuromuscular junction. Here, we adopted ELISA and flow cytometry techniques to measure the levels of Th1, Th2, Th3 cytokines, inflammatory cytokine and chemokine sICAM-1 and to analyze the phenotypes of $CD4^+$ and $CD8^+$ regulatory cells as well as the expression of BAFF-R on $CD19^+$ B cells in peripheral blood from 75 MG patients and 50 healthy controls. There were no differences in the levels of IL-2, IL-4, IL-10, IL-13, IFN-γ, TNF-α, TGF-β and sCTLA-4 in both sera and culture supernatants between MG patients and healthy controls. The level of IL-12 was decreased in culture supernatants from MG patients, and the level of sICAM-1 was increased in both sera and culture supernatants from MG patients. Although the populations of $CD8^+CD28^-$ and $CD8^+CD122^+$ regulatory T cells were not different between MG patients and healthy controls, MG patients exhibited the decrease of $CD4^+CD25^{high}Foxp3^+$ regulatory T cells and the increase of $CD19^+BAFF\text{-}R^+$ B cells, revealing that MG patients should display the dysfunction of T cell balance and the activation of B cell maturation.

Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by the failure of neuromuscular transmission, which results from the binding of autoantibodies to acetylcholine receptor (AChR) at the neuromuscular junction (NMJ) [1]. Although the cause of this disorder is unknown, the mechanism of immune response (especially for circulating antibodies against AChR) in its pathogenesis is well established in patients with MG [1]. However, many studies have demonstrated that both Th1 and Th2 lymphocytes and cytokines probably participate in the development of MG [2]. $CD4^+$ T cells specific for AChR are required for the development of experimental autoimmune MG (EAMG) [3]. Clinical EAMG was nearly completely prevented in $CD4^-8^-$, $CD4^{-/-}$, and $CD8^{-/-}$ mice [4]. A persistent clonally expanded $CD4^+$ helper T cell population was observed in the blood of MG patients [5]. AChR α146–162 peptide-specific T cells from AChR-immunized mice

* Corresponding author.
E-mail address: lujiahong@yahoo.com (J.-H. Lu).

1521-6616/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2007.10.001

Table 1 General characteristics of MG patients in this study

Severity of disease	Number of patients (%)	M/F
I	19 (25.3)	4/15
IIa	30 (40.0)	7/23
IIb	21 (28.0)	16/5
III	5 (6.7)	2/3
Sera antibody		
Positive	53 (70.7)	21/32
Negative	22 (29.3)	8/14
Disease duration		
<6 months	32 (42.7)	10/22
6 months–3 years	29 (38.7)	8/21
3 years–5 years	4 (5.3)	3/1
>5 years	10 (13.3)	8/2
Thymus histopathology		
Thymoma	25 (33.3)	12/13
Hyperplasia	31 (41.3)	9/22
Normal	19 (25.4)	8/11
Thymectomy		
<3 months	18 (24.0)	10/8
3–6 months	4 (5.3)	1/3
>6 months	14 (18.7)	6/8

help AChR-specific B cells to produce autoantibodies against the mouse AChR [6], indicating that the pathogenesis of this disease results from the collaborative effects of T and B cells and subsequent activity of autoantibodies and complements.

Both AChR-specific Th1 and Th2 cells are documented in MG patients, but it is not yet clear which are essential for driving the pathogenesis of MG patients. Th1 cytokines are important in cell-mediated immune responses, whereas Th2 cytokines are important inducers of humoral immune responses. However, Th2 cells have complex and contrasting roles in EAMG [2,7–10].

Intercellular adhesion molecule-1 (ICAM-1; CD54) has long been implicated in the pathogenesis of multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), but little is known about the regulation of ICAM-1 in the pathogenesis of MG. Recent studies have identified ICAM-1 as a co-stimulatory ligand that binds to lymphocyte function associated antigen-1 (LFA-1), thereby promoting the activation of T cells. On the other hand, LFA-1/ICAM-1 interaction declines the threshold for B cell activation by promoting B cell adhesion and synapse formation [11], confirming the important role of ICAM-1 as a signaling molecule in B cell activation [12]. Soluble ICAM-1 (sICAM-1) may serve as a serologic marker of disease activity in patients with systemic lupus erythematosus (SLE) [13], rheumatoid arthritis [14], connective tissue diseases [15], Graves' disease [16], chronic hepatitis C[17] and MG [18].

We aimed to investigate the status of immune responses in MG patients by exploring the levels of Th1, Th2, Th3 cytokines, inflammatory cytokines and chemokine sICAM-1 and analyzing the phenotypes of $CD4^+$ and $CD8^+$ regulatory cells as well as the expression of BAFF-R on $CD19^+$ B cells.

Materials and methods

Study subjects

Seventy-five unselected MG patients from myasthenia outpatient clinics and neurological wards at Huashan Hospital, Shanghai, China were recruited in this study. The diagnosis of MG was based on clinical symptoms, positive response to cholinesterase inhibitors, abnormal results in the neurophysiological tests and, in most patients, presence of serum anti-AChR antibodies. Disease duration and the disease flare (severity of disease, thymus histopathology and immunotherapy) at the time of blood sampling are shown in Table 1, Table 2. The mean age of the 75 MG patients was 36 years (ranging from 5 to 66 years). Fifty healthy volunteers (mean age = 32, ranging from 16 to 53 years) were selected at random from an available set of normal data to achieve age and sex matching. Healthy donors had not taken any medications for at least 4 weeks. Serum samples were collected from MG patients and healthy donors, and kept in −70 °C until analysis.

Isolation and culture of mononuclear cells

Peripheral blood mononuclear cells (MNC) from heparinized blood were separated by density gradient centrifugation over Ficoll/Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Cells from the interface were washed with PBS and adjusted to a concentration of 2×10^6/ml in Dulbecco's modified Eagle's minimal essential medium (Gibco, Paisley,

Table 2 Immunotherapies of MG patients in this study

Treatment	*N* (%)	Dosage (*n*)	
No treatment	12 (16.0)		
Corticosteroid (iv)	9 (12.0)	500 mg/day	
Corticosteroid and Ig (iv)	8 (10.7)	500 mg/day and 400 mg/kg/day	
Ig (iv)	13 (17.3)	400 mg/kg/day	
Corticosteroid (oa)	7 (9.3)	≤50 mg/day (5)	>50 mg/day (2)
Aza (oa)	7 (9.3)	25 mg bid (3)	50 mg bid (4)
Corticosteroid + Aza (oa)	19 (25.4)	≤50 mg/day + 50 mg bid	

Aza = azathioprine.
oa = oral administration.
iv = intravenous.
N/*n* = number of patients.

UK) supplemented with 10% heat-inactivated FBS (Gibco), antibiotics 200 U of penicillin (Gibco). Trypan blue exclusive assay was used to exclude any cell death. Cells (2×10^6/ml) were cultured in complete medium at 37 °C for 5% CO_2. After 48 h, supernatants were collected for cytokine measurement, and cells were harvested for flow cytometry.

Measurement of cytokines and chemokines by ELISA

IL-2, IL-4, IL-10, IL-12, IL-13, IFN-γ, TNF-α, TGF-β, sICAM-1 and sCTLA-4 were detected by using human ELISA kits from BD Bioscience Pharmingen following the manufacturer's instructions. The levels of cytokines were quantified by reference to

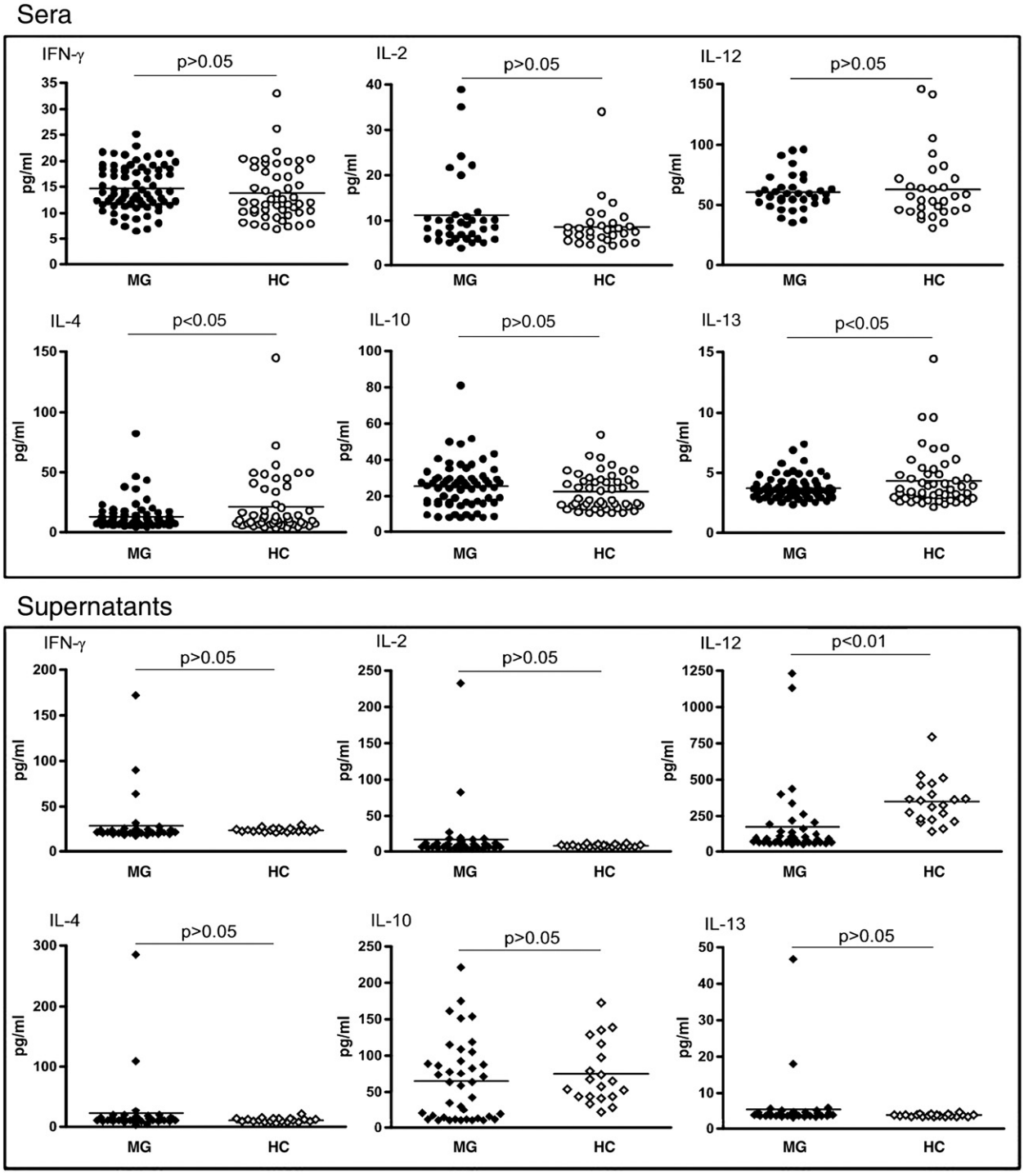

Figure 1 The levels of Th1 and Th2 cytokine concentrations in sera and culture supernatants from MG patients (MG) and healthy controls (N). The concentration of cytokines in sera and culture supernatants was determined by ELISA. The *y*-axis shows cytokine concentrations (pg/ml). Each dot represents the mean value of duplicates. The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

standard curves. Determinations were performed in duplicate and results were expressed as pg/ml.

Flow cytometry

For phenotypic analysis, MNC were stained with the following panel of antibodies: FITC-CD4, PE-CD25, FITC-CD8, PE-CD28, PE-CD122, TC-CD19 and PE-BAFF-R (BD Biosciences, San Diego, CA) for 20 min at 4 °C in 1% BSA-PBS buffer, washed twice with PBS and analyzed using a Coulter Epics XL flow cytometer (Becton Dickinson, Mountain View, CA). Gates were set so that the $CD4^{+}CD25^{-}$ population was based on the isotype control while the $CD25^{high}$ population was determined relative to the low intensity of CD25 staining. Intracellular

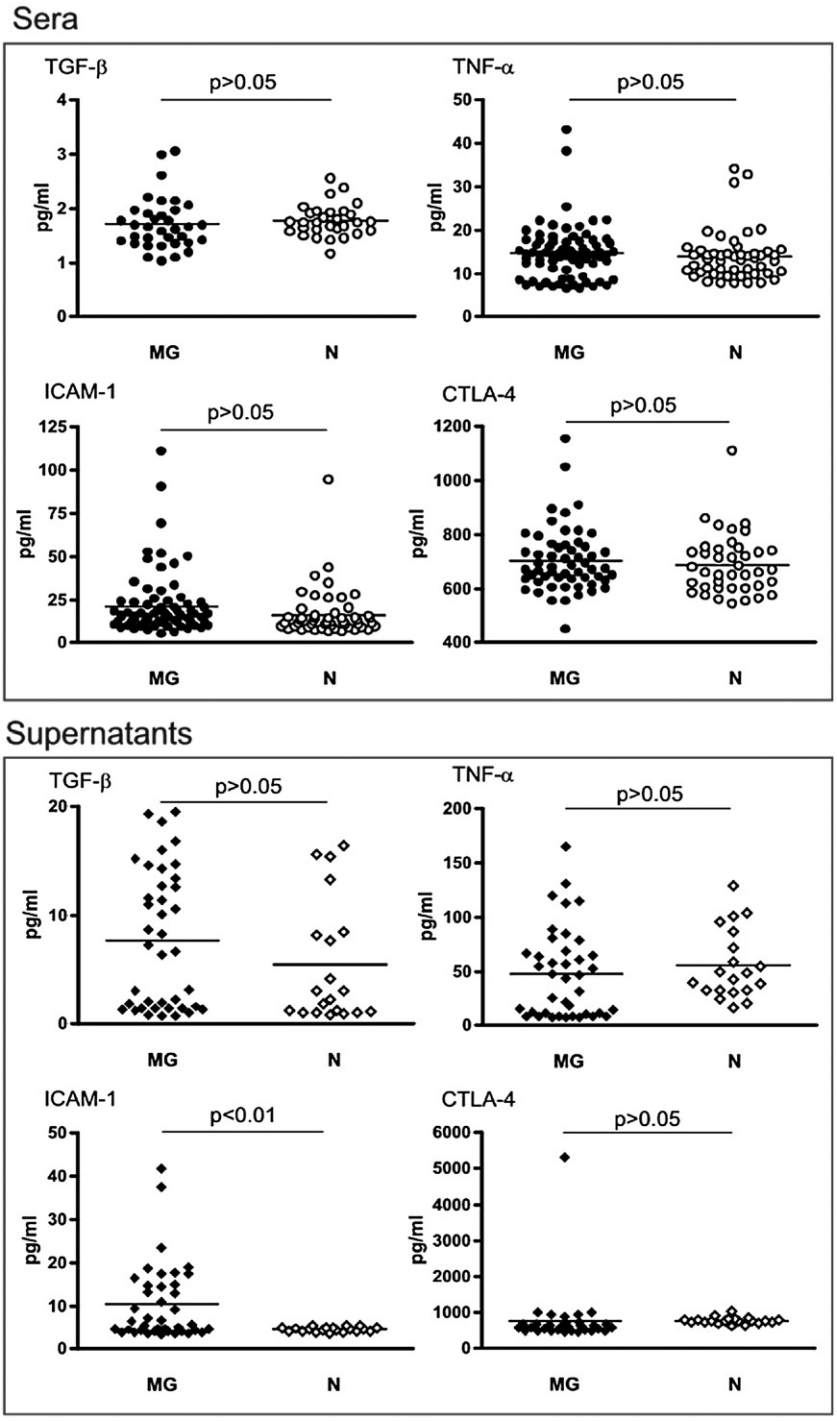

Figure 2 The levels of TGF-β, TNF-α and soluble ICAM-1 and CTLA-4 concentrations in sera and culture supernatants from MG patients (MG) and healthy controls (N). The concentration of cytokines in sera and culture supernatants was determined by ELISA. The *y*-axis shows cytokine concentrations (pg/ml). Each dot represents the mean value of duplicates. The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

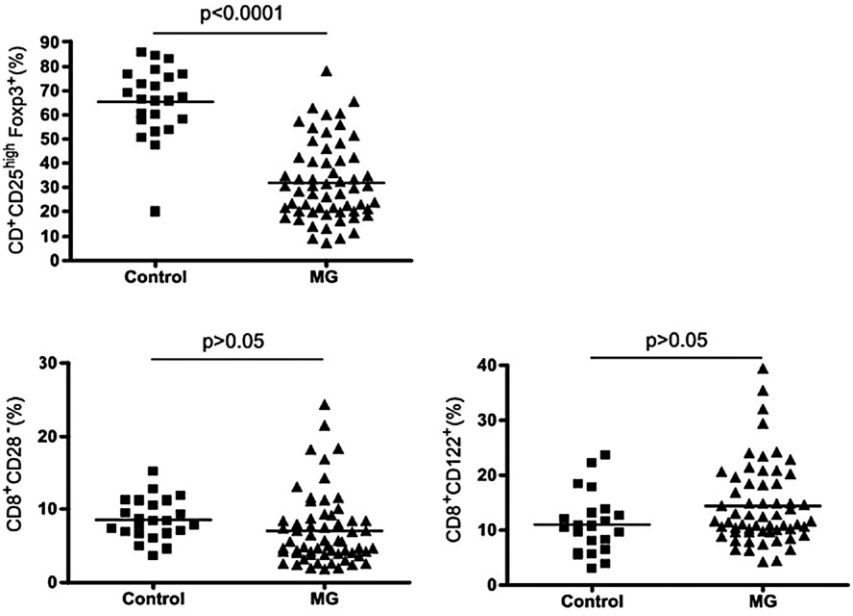

Figure 3 The populations of CD4 and CD8 regulatory T cells in peripheral blood from MG patients and healthy controls. The populations of CD4 and CD8 regulatory T cells were determined by flow cytometry. The *y*-axis shows percentages of regulatory T cells (%). The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

Foxp3 staining was performed with PE-Cy5-Foxp3 in 1× Permeabilization Buffer (eBioscience Inc., San Diego). In brief, MNC were washed in PBS, fixed in 1 ml of PBS with 1% paraformaldehyde for 20 min at 4 °C. Cells were incubated with PE-Cy5-Foxp3.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) statistical software package. Comparisons between the two groups were made using the Student's *t* test. *p* values less than 0.05 were considered significant (two-tailed).

Results

Soluble factors in sera and culture supernatants from MG and healthy controls

We detected the levels of Th1 and Th2 cytokines in sera and culture supernatants from MG patients and healthy controls. MG patients had reduced levels of IL-12 ($p<0.01$) in sera and did not demonstrate any difference for IFN-γ, IL-2, IL-4, IL-10 and IL-13 in both sera and culture supernatants (Fig. 1). We also measured the levels of TGF-β, TNF-α, sICAM-1 and sCTLA-1 in both sera and culture supernatants from healthy controls and MG patients. Only the level of sICAM-1 was elevated in culture supernatants from MG patients ($p<0.01$) and did not show a significant increase in sera ($p=0.054$) (Fig. 2).

Regulatory T cells from healthy controls and MG patients

Because regulatory T cell population was shown to play an important role in the maintenance of peripheral tolerance, we measured the levels of regulatory T cells including $CD4^+CD25^{high}Foxp3^+$, $CD8^+CD28^-$ and $CD8^+CD122^+$ T cells. We found that MG patients had significantly lower numbers of $CD4^+CD25^{high}Fosp3^+$ T cells than healthy subjects ($p<0.0001$) (Fig. 3). The intensity of $CD8^+CD28^-$ and $CD8^+CD122^+$ regulatory T cell population was not statistically different in MG patients compared to healthy controls ($p>0.05$, respectively) (Fig. 3).

$CD19^+BAFF\text{-}R^+$ B cells in MG patients and healthy controls

In MG patients, AChR situated at the neuromuscular endplate are destroyed by autoantibodies against AChR. BAFF–BAFF receptor signal pathway on B cells is essential for B-cell survival and activation. To assess whether the intensity of BAFF-R expression on B cells was different in MG patients compared to healthy controls, we measured the intensity of

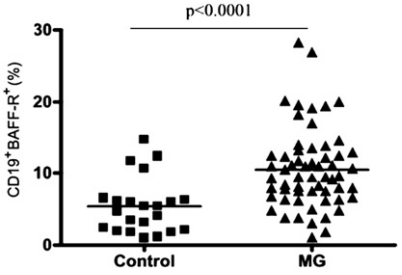

Figure 4 The populations of $CD19^+BAFF\text{-}R^+$ B cells in peripheral blood from MG patients and healthy controls. The populations of $CD19^+BAFF\text{-}R^+$ B cells were determined by flow cytometry. The *y*-axis shows percentages of $CD19^+BAFF\text{-}R^+$ B cells (%). The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

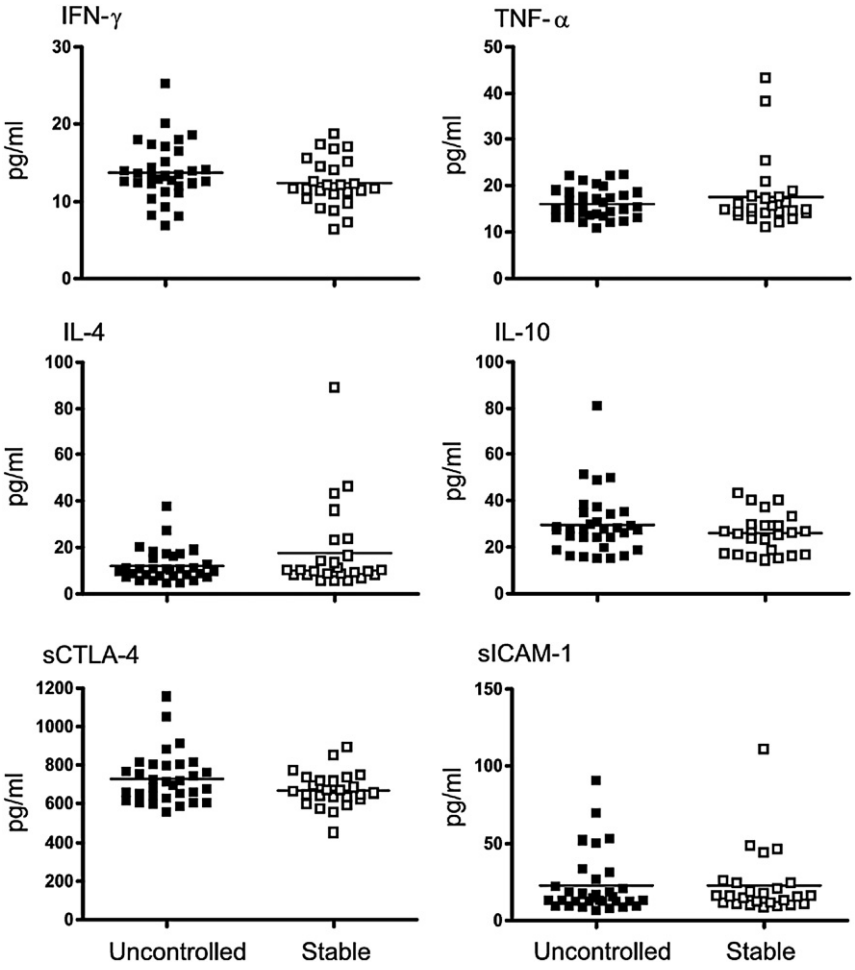

Figure 5 The comparison of IFN-γ, TNF-α, IL-4, IL-10, sCTLA-4 and sICAM-1 concentrations in sera from uncontrolled and stable of MG patients. The concentration of cytokines and chemokine in sera was determined by ELISA. The *y*-axis shows cytokine concentrations (pg/ml). Each dot represents the mean value of duplicates. The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

BAFF-R on $CD19^+$ B cells. We detected a significant increase of BAFF-R^+CD19^+ B cells ($p<0.0001$) in MG patients compared to healthy controls (Fig. 4). We have checked the levels of IgG in sera and did not find the correlation between the numbers of $CD19^+BAFFR^+$B cells and the levels of IgG (data not shown).

Comparison of soluble cytokines and chemokine at different phase and after corticosteroid therapy

We compared the levels of IFN-γ, TNF-α, IL-4, IL-10, sCTLA-4 and sICAM-1 concentrations in sera from MG patients at stable and uncontrolled phase of disease. As seen in Fig. 5, no significant differences were observed between stable and uncontrolled phase in MG patients ($p>0.05$). In parallel comparison, we also tested the effects of corticosteroid therapy (1 month) in MG patients. Results demonstrate that corticosteroid therapy did not change the levels of IFN-γ, TNF-α, IL-4, IL-10, sCTLA-4 and sICAM-1 concentrations in sera (Fig. 6). Taken together, the results reveal that these soluble factors may be not necessary for the pathogenesis and the improvement of MG.

Discussion

Measurement of cytokines and/or soluble markers is useful for understanding pathogenesis and as diagnostic and prognostic indicators in many autoimmune diseases [19]. Although Th1 and Th2 cytokines seem to be important for the development of EAMG and MG patients, these molecules in the present study were not different between MG patients and normal controls. The complications and difficulties in cytokine assays have been reported [20–22], and a number of factors have been shown to affect the quality and validity of the measurement [23 24]. It should be considered that measurement of *in vivo* cytokine production might be problematic owing to the rapid utilization, catabolization and excretion of cytokine quickly after production and secretion. In addition, a2-microglobulin, autoantibodies and soluble cytokine receptors bind cytokines and affect the recognition of cytokines by immunoassay [22]. Based on these considerations, we measured serum cytokine concentration and spontaneous cytokine production in culture supernatants. Beside increase of ICAM-1 production in patients with MG, the levels of Th1/Th2/Th3 cytokines, sCTLA-4 and

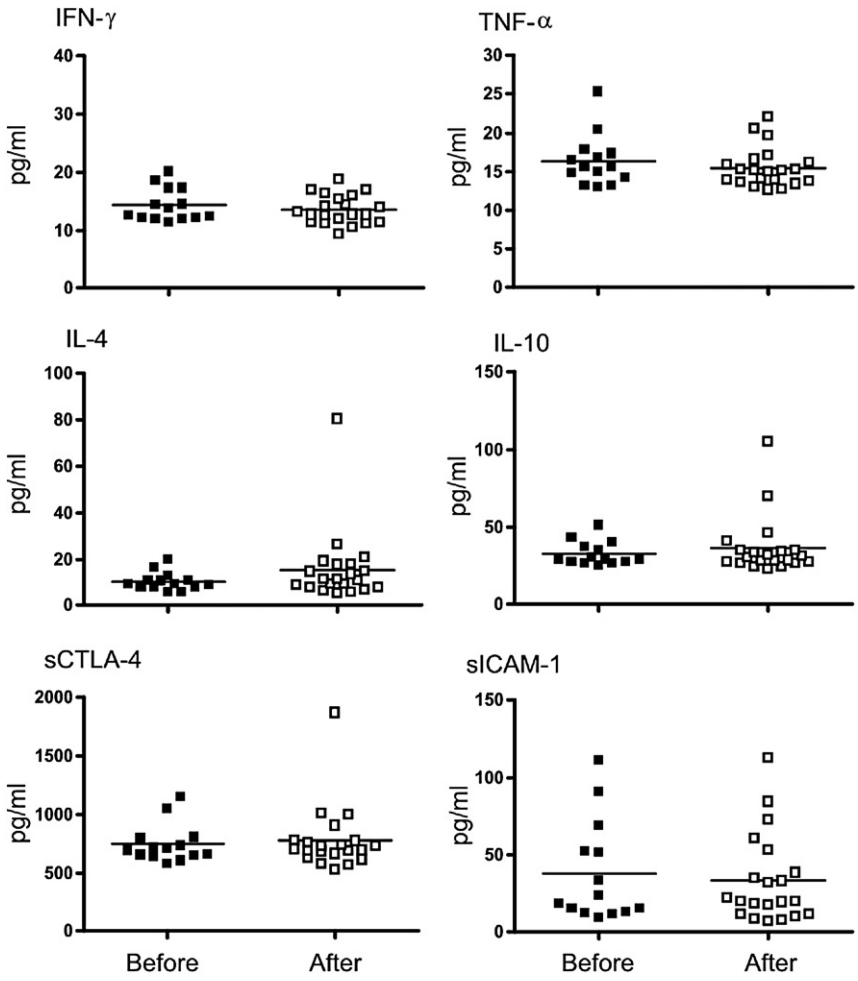

Figure 6 The comparison of IFN-γ, TNF-α, IL-4, IL-10, sCTLA-4 and sICAM-1 concentrations in sera from MG patients before and after corticosteroid therapy. The concentration of cytokines and chemokine in sera was determined by ELISA. The *y*-axis shows cytokine concentrations (pg/ml). Each dot represents the mean value of duplicates. The bar represents the mean value and *p* values less than 0.05 were considered significant (two-tailed).

TNF-α are not significantly altered in MG patients compared to healthy controls.

To prevent autoimmune diseases induced directly by autoimmune attack, a complex network of immunity and tolerance mechanism has co-evolved to prevent or dampen immune-mediated diseases. Distinct subsets of regulatory $CD4^+$ and $CD8^+$ T cells have emerged as important cells in the induction of peripheral tolerance. The present study explored both $CD4^+$ and $CD8^+$ T cell subsets in the peripheral blood of patients with MG. We found that $CD4^+CD25^{high}Foxp3^+$ cells are dramatically decreased in the peripheral blood of patients with MG compared to healthy controls. However, a remarkable decrease of $CD4^+CD25^{high}Foxp3^+$ cells was not associated with decreased IL-10 and/or TGF-β levels in MG patients. The capacity of $CD4^+CD25^{high}Foxp3^+$ cells to produce immunosuppressive or immunomodulatory cytokines remains controversial. Whereas some studies claim that $CD4^+CD25^+$ cells do not produce cytokines [25,26], others demonstrate that these cells may secrete cytokines, including IL-10 and/or TGF-β [27,28]. While $CD4^+$ T lymphocytes have recently attracted the most attention, $CD8^+$ regulatory T cell populations are also believed to play an important role in control of autoimmunity. Some $CD8^+$ T cell lineages have also been suggested as regulatory T cells [29–31]. In our study, there was no difference on circulating $CD8^+CD28^-$ and $CD8^+CD122^+$ T cells between MG patients and healthy controls.

The BAFF–BAFF-R axis appears to be the most crucial pathway that transduces survival signals to B cells [32]. We found that the frequency of BAFF-R expression on $CD19^+$ B cells was statistically increased in MG patients compared with healthy controls. Ng et al. reported that activated T cells up-regulated BAFF-R surface expression [33]. A remarkable decrease of $CD4^+CD25^{high}Foxp3^+$ cells should reveal a relative activation of T cells that may induce the up-regulation of BAFF-R expression on $CD19^+$ B cells. The ability of self-reactive B cells to be rescued by BAFF is most likely determined by their expression of BAFF-R, which peaks around the point during B cell maturation where BAFF-mediated rescue begins to operate [34]. It will be of great interest to determine whether BAFF/BAFF-R signaling might be associated with human MG.

ICAM-1 is a member of the immunoglobulin supergene family and plays a central role in cell-to-cell and in cell-to-extracellular matrix-mediated immune responses and has been advocated as a parameter of clinical relevance in determining the activation of the immune system in autoimmune

diseases. Elevated levels of sICAM-1 were observed in rheumatoid arthritis [14], HIV-1 infection [35], chronic hepatitis C [36] and systemic lupus erythematosus [37]. In our study, a tendency to increased levels of sICAM-l in serum was observed in MG patients, although it did not reach statistical significance ($p = 0.054$). However, the levels of sICAM-1 were increased significantly in culture supernatants from MG patients. Noteworthy, high levels of sICAM-1 were detected in multiple sclerosis (MS) patients benefiting from interferon beta treatment [38,39]. The levels of sICAM-1 were also elevated in FK506-treated NOD mouse [40]. Patients with MG treated by plasma exchange also exhibited high levels of sICAM-1 [41]. These results indicate that stabilization of the expression of cell surface bound ICAM-1 on blood MNC may indicate the beneficial effects of sICAM-1 after drug therapy in MS patients [39], revealing that sICAM 1 might have a relevant immunoregulatory role in autoimmune diseases.

In summary, there was no difference in the levels of IL-2, IL-4, IL-10, IL-13, IFN-γ, TNF-α, TGF-β and sCTLA-4 detected in sera and culture supernatants between MG patients and healthy controls. The levels of IL-12 were decreased in sera from MG patients, and the levels of sICAM-1 were increased in sera from MG patients. MG patients exhibited decreased population of $CD4^+CD25^{high}Foxp3^+$ regulatory T cells followed by increased $CD19^+BAFF\text{-}R^+$ B cells, revealing that MG patients should display the dysfunction of T cell balance and the activation of B cell maturation.

Acknowledgments

This work was supported by project grants from the National Natural Science Foundation of China (Code: 30571727) and Shanghai Key Discipline (Neurology) Construction Found.

References

[1] B.M. Conti-Fine, M. Milani, H.J. Kaminski, Myasthenia gravis: past, present, and future, J. Clin. Invest. 116 (11) (2006) 2843–2844.
[2] M. Milani, N. Ostlie, W. Wang, B.M. Conti-Fine, T cells and cytokines in the pathogenesis of acquired myasthenia gravis, Ann. N. Y. Acad. Sci. 998 (2003) 284–287.
[3] L. Xu, M. Villain, F.S. Galin, S. Araga, J.E. Blalock, Prevention and reversal of experimental autoimmune myasthenia gravis by a monoclonal antibody against acetylcholine receptor-specific T cells, Cell Immunol. 208 (2) (2001) 107–4.
[4] G.X. Zhang, B.G. Xiao, M. Bakhiet, M.P. van, H. Wigzell, H. Link, T. Olsson, Both CD4+ and CD8+ T cells are essential to induce experimental autoimmune myasthenia gravis, J. Exp. Med. 184 (2) (1996) 349–56.
[5] B. Tackenberg, J. Kruth, J.E. Bartholomaeus, K. Schlegel, W.H. Oertel, N. Willcox, B. Hemmer, N. Sommer, Clonal expansions of CD4+ B helper T cells in autoimmune myasthenia gravis, Eur. J. Immunol. 37 (3) (2007) 849–63.
[6] J.S. Rosenberg, M. Oshima, M.Z. Atassi, B-cell activation in vitro by helper T cells specific to region alpha 146–162 of *Torpedo californica* nicotinic acetylcholine receptor, J. Immunol. 157 (7) (1996) 3192–3199.
[7] M.A. Poussin, E. Goluszko, J.U. Franco, P. Christadoss, Role of IL-5 during primary and secondary immune response to acetylcholine receptor, J. Neuroimmunol. 125 (1–2) (2002) 51–58.
[8] G.X. Zhang, B.G. Xiao, L.Y. Yu, M.P. van, H. Link, Interleukin 10 aggravates experimental autoimmune myasthenia gravis through inducing Th2 and B cell responses to AChR, J. Neuroimmunol. 113 (1) (2001) 10–18.
[9] N.S. Ostlie, P.I. Karachunski, W. Wang, C. Monfardini, M. Kronenberg, B.M. Conti-Fine, Transgenic expression of IL-10 in T cells facilitates development of experimental myasthenia gravis, J. Immunol. 166 (8) (2001) 4853–62.
[10] C. Deng, E. Goluszko, E. Tuzun, H. Yang, P. Christadoss, Resistance to experimental autoimmune myasthenia gravis in IL-6-deficient mice is associated with reduced germinal center formation and C3 production, J. Immunol. 169 (2) (2002) 1077–83.
[11] Y.R. Carrasco, S.J. Fleire, T. Cameron, M.L. Dustin, F.D. Batista, LFA-1/ICAM-1 interaction lowers the threshold of B cell activation by facilitating B cell adhesion and synapse formation, Immunity 20 (5) (2004) 589–99.
[12] J. Holland, T. Owens, Signaling through intercellular adhesion molecule 1 (ICAM-1) in a B cell lymphoma line. The activation of Lyn tyrosine kinase and the mitogen-activated protein kinase pathway, J. Biol. Chem. 272 (14) (1997) 9108–12.
[13] R.A. Sari, S. Taysi, F. Erdem, O. Yilmaz, S. Keles, A. Kiziltunc, A.R. Odabas, R. Cetinkaya, Correlation of serum levels of soluble intercellular adhesion molecule-1 with disease activity in systemic lupus erythematosus, Rheumatol. Int. 21 (4) (2002) 149–152.
[14] A.J. Potocnik, R. Kinne, H. Menninger, J. Zacher, F. Emmrich, R.A. Kroczek, Expression of activation antigens on T cells in rheumatoid arthritis patients, Scand. J. Immunol. 31 (2) (1990) 213–214.
[15] A. Kuryliszyn-Moskal, P.A. Klimiuk, S. Sierakowski, Serum autoantibodies profile and increased levels of circulating intercellular adhesion molecule-1: a reflection of the immunologically mediated systemic vasculopathy in rheumatic diseases? Arch. Immunol. Ther. Exp. (Warsz) 49 (6) (2001) 423–430.
[16] E. Sonnet, C. Massart, J. Gibassier, H. Allannic, D. Maugendre, Longitudinal study of soluble intercellular adhesion molecule-1 (ICAM-1) in sera of patients with Graves' disease, J. Endocrinol. Invest. 22 (6) (1999) 430–435.
[17] A.M. El-Gohary, N.A. Fawaz, H.M. Hassoba, A. Serwah, M. Ali, Soluble ICAM-1 in patients with chronic hepatitis C infection: a prognostic marker of disease activity, Egypt J. Immunol. 11 (2) (2004) 109–19.
[18] M. Kakoulidou, X. Wang, X. Zhao, R. Pirskanen, A.K. Lefvert, Soluble costimulatory factors sCD28, sCD80, sCD86 and sCD152 in relation to other markers of immune activation in patients with myasthenia gravis, J. Neuroimmunol. 185 (1–2) (2007) 150–151.
[19] L.J. Lesko, A.J. Atkinson, Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies, Annu. Rev. Pharmacol. Toxicol. 41 (2001) 347–66.
[20] J.G. Cannon, J.L. Nerad, D.D. Poutsiaka, C.A. Dinarello, Measuring circulating cytokines, J. Appl. Physiol. 75 (4) (1993) 1897–902.
[21] R. Thorpe, M. Wadhwa, C.R. Bird, A.R. Mire-Sluis, Detection and measurement of cytokines, Blood Rev. 6 (3) (1992) 133–138.
[22] J. Whicher, E. Ingham, Cytokine measurements in body fluids, Eur. Cytokine Netw. 1 (4) (1990) 239–43.
[23] N. Aziz, P. Nishanian, J.L. Fahey, Levels of cytokines and immune activation markers in plasma in human immunodeficiency virus infection: quality control procedures, Clin. Diagn. Lab. Immunol. 5 (6) (1998) 755–61.
[24] C.A. Dinarello, ELISA kits based on monoclonal antibodies do not measure total IL-1 beta synthesis, J. Immunol. Methods 148 (1–2) (1992) 255–259.

[25] M.G. Roncarolo, S. Gregori, M. Levings, Type 1 T regulatory cells and their relationship with CD4+CD25+ T regulatory cells, Novartis Found Symp. 252 (2003) 115–152.
[26] H. Grindebacke, K. Wing, A.C. Andersson, E. Suri-Payer, S. Rak, A. Rudin, Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season, Clin. Exp. Allergy 34 (9) (2004) 1364–72.
[27] S. Sakaguchi, Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses, Annu. Rev. Immunol. 22 (2004) 531–532.
[28] C. Baecher-Allan, V. Viglietta, D.A. Hafler, Human CD4+CD25+ regulatory T cells, Semin. Immunol. 16 (2) (2004) 89–98.
[29] N. Suciu-Foca, J.S. Manavalan, R. Cortesini, Generation and function of antigen-specific suppressor and regulatory T cells, Transpl. Immunol. 11 (3–4) (2003) 235–44.
[30] N. Najafian, T. Chitnis, A.D. Salama, et al., Regulatory functions of CD8+CD28− T cells in an autoimmune disease model, J. Clin. Invest. 112 (7) (2003) 1037–1038.
[31] H. Suzuki, Y.W. Zhou, M. Kato, T.W. Mak, I. Nakashima, Normal regulatory alpha/beta T cells effectively eliminate abnormally activated T cells lacking the interleukin 2 receptor beta in vivo, J. Exp. Med. 190 (11) (1999) 1561–1562.
[32] J.S. Thompson, S.A. Bixler, F. Qian, et al., BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF, Science 293 (5537) (2001) 2108–11.
[33] L.G. Ng, A.P. Sutherland, R. Newton, et al., B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells, J. Immunol. 173 (2) (2004) 807–17.
[34] M. Thien, T.G. Phan, S. Gardam, M. Amesbury, A. Basten, F. Mackay, R. Brink, Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches, Immunity 20 (6) (2004) 785–788.
[35] J. Most, R. Zangerle, M. Herold, D. Fuchs, H. Wachter, P. Fritsch, M.P. Dierich, Elevated concentrations of circulating intercellular adhesion molecule 1 (ICAM-1) in HIV-1 infection, J. Acquir. Immune Defic. Syndr. 6 (3) (1993) 221–226.
[36] A.M. El-Gohary, N.A. Fawaz, H.M. Hassoba, A. Serwah, M. Ali, Soluble ICAM-1 in patients with chronic hepatitis C infection: a prognostic marker of disease activity, Egypt J. Immunol. 11 (2) (2004) 109–19.
[37] K. Egerer, E. Feist, U. Rohr, A. Pruss, G.R. Burmester, T. Dorner, Increased serum soluble CD14, ICAM-1 and E-selectin correlate with disease activity and prognosis in systemic lupus erythematosus, Lupus 9 (8) (2000) 614–21.
[38] M. Giorelli, B.A. De, G. Defazio, C. Avolio, L. Iacovelli, P. Livrea, M. Trojano, Differential regulation of membrane bound and soluble ICAM 1 in human endothelium and blood mononuclear cells: effects of interferon beta-1a, Cell Commun. Adhes. 9 (5–6) (2002) 259–72.
[39] J. Kraus, R. Bauer, N. Chatzimanolis, et al., Interferon-beta 1b leads to a short-term increase of soluble but long-term stabilisation of cell surface bound adhesion molecules in multiple sclerosis, J. Neurol. 251 (4) (2004) 464–2.
[40] G. Papaccio, M.V. Latronico, A. Graziano, A. Lanza, M. Pedulla. Tacrolimus, but not cyclosporine A, significantly increases expression of ICAM-1 and IFN-gamma in the NOD mouse. J. Cell Biochem. Suppl. 2001; Suppl 36:107–6.
[41] V. Tesar, E. Jelinkova, M.J. Jirsa, M. Bakosova, P. Pitha, V. Chabova, Soluble adhesion molecules and cytokines in patients with myasthenia gravis treated by plasma exchange, Blood Purif. 18 (2) (2000) 115–120.