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The increased expression of CD21 on AchR specified B cells in patients with myasthenia gravis

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ABSTRACT

CD21, a major complement receptor expressed on B cells, is associated with autoimmune disorders. In the present study, we investigated the role of CD21 in pathogenesis of myasthenia gravis (MG) in relationship to anti-acetylcholine receptor (AchR) IgG (anti-AchR IgG) secretion. We detected increased surface expression of CD21 on AchR specified B cells as well as decreased surface expression of CD21 on total B cells in peripheral blood of patients with generalized MG (gMG). In addition, the serum concentrations of soluble secreted CD21 (sCD21) were decreased in patients with gMG. We also found that the level of $CD21^+$ AchR specified B cells correlated positively with serum anti-AchR IgG level, while the serum concentration of soluble CD21 correlated negatively with serum anti-AchR IgG level. Our data suggests that CD21 might facilitate its function on AchR specified B cell activation, resulting in the secretion of anti-AchR IgG.

1. Introduction

Myasthenia gravis (MG), a disease with impaired neuromuscular transmission, is a T cell dependent, antibody and complement mediated autoimmune disease (Turner, 2007). MG patients usually have clinical manifestations of fatigue and fluctuating weakness of voluntary muscles (McGrogan et al., 2010). The pathogenesis of MG involves genetic and environmental factors which cause aberrant generation of autoreactive antibodies against acetylcholine receptor (AchR) (McGrogan et al., 2010). Approximately 85–90% of autoreactive antibodies in MG are anti-AchR antibodies (Bateman et al., 2007). The antibodies bind with AchRs in the neuromuscular junction (NMJ), activate the complement system, and lead to postsynaptic membrane damage as well as accelerating the degradation of the AchRs thus leading to fatigue of voluntary muscles (Tüzün et al., 2008; Souroujon et al., 2010; Tüzün et al., 2010). Alexa-conjugated AChR was used as a probe for AchR specified B cells ($B220^+Ig^+$). Mice with experimental autoimmune myasthenia gravis (EAMG) had significantly elevated frequencies of AchR specified $IgG2^+$ (IgG1 in human) and IgM^+ B cells. The frequencies of AchR specified B cells significantly correlated with the clinical grade of disease and loss of limb muscle strength (Allman et al., 2011). These results indicated that significantly elevated frequencies of AchR specified peripheral blood B cells could be a potential biomarker for MG disease severity. However, there are no studies on the characterization of AchR specified B cells in the periphery of MG patients.

The complement system, which plays a pivotal role in innate and adaptive immune responses (Holers, 2005; Isaák et al., 2006; Holers and Kulik, 2007; Roozendaal and Carroll, 2007), has been investigated extensively in MG (Wagner and Frank, 2010). In the mouse model of EAMG, complements, such as C3 and C5b–C9, were accumulated on the postsynaptic membrane of NMJ, and more the C5b–C9 deposits less the functional AchR (Tüzün et al., 2003; Tüzün et al., 2004; Christadoss et al., 2008). Previous study has shown that membrane damage of NMJ was initiated by anti-AchR antibody mediated complement damage (Tüzün et al., 2003; Tüzün et al., 2004; Christadoss et al., 2008). Complements exert their effects through binding with complement receptors (CRs). CRs, including CR1, CR2, CR3, CR4, are expressed widely on immunocytes, such as T lymphocytes, B lymphocytes, dendritic cells (Fischer et al., 1991; Roozendaal and Carroll, 2007; Erdei et al., 2009; Twohig et al., 2009). CD21, also called CR2, mainly expressed on B lymphocytes, is responsible for B cell survival and activation (Erdei et al., 2009; Dunkelberger and Song, 2010). CD21 on peripheral B cells could reduce the threshold of B cell activation, stop surface IgM (sIgM) mediated B cell apoptosis, favor antigen presentation, stabilize BCR "flip-flop", regulate the expression of costimulatory molecules, and transport immune complexes (ICs) to germinal centers (Patterson et al., 2006; Dunkelberger and Song, 2010). Recently, several lines of evidence demonstrated that the levels of CD21 were decreased on serum and synovial B cells of patients suffering from systemic lupus erythematosus or rheumatoid

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arthritis (Wehr et al., 2004; Patterson et al., 2006; Isaák et al., 2008). It has also been found that CD21 may be correlated positively with the maturity of newborn immune system, which further helps to explain the feasibility of infectious diseases in newborns (Zhang et al., 2007). All the evidence suggests that CD21 has a close relationship with immune disorders.

Until now, there are no report regarding the relationship of CD21 and MG. In the present study, we aimed to characterize the AchR specified B cells and investigated the role of CD21 in immunopathogenesis of MG. We utilized the Allophycocyanin (APC)-conjugated AchR to measure the frequencies of AchR specified B cells in the periphery of MG patients, and further detected the CD21 expression on AchR specified B cells. We found that the levels of $CD21^+$ AchR specified B cells correlated positively with serum anti-AchR IgG level, while the serum concentration of soluble CD21 correlated negatively with serum anti-AchR IgG level. Our data suggests that CD21 might facilitate its function on AchR specified B cell activation, resulting in the secretion of anti-AchR IgG.

2. Materials and methods

2.1. Subjects

Twenty generalized MG (gMG) patients were enrolled for this study in Xiangya Hospital during January 2009 to March 2010. All patients were diagnosed based on the clinical symptoms, neostigmine test, and electrophysiological examination. Those who had taken glucocorticoids or immunosuppressants in the last 3 months or had any other autoimmune diseases were excluded from this study. The patients enrolled were 8 males and 12 females with ages ranging from 16–60 years old. During the experimental period, none of patients had undergone thymectomy. All gMG patients were examined for serum anti-AchR IgG, and 17 patients were found positive. The enrolled gMG patients were classified into subgroups IIa and IIb with the quantitative MG scores ranging from 10 to 27 (mean 17.6) according to Myasthenia Gravis Foundation of America criteria (Barohn, 2003). Also 20 age and gender matched healthy controls (HCs) (8 males, 12 females; age 16–60 years old) were included in the study. A total of 17 ml blood was collected from each participant. Three ml was used for flow cytometry, 7 ml was used for ELISA, and 10 ml was used for RT-PCR. The present study was approved by the ethics committee of the University and adhered to the guidelines of the World Medical Association's declaration of Helsinki. All participants were informed about the study and gave consent (Table 1).

2.2. Flow cytometry

Plasma is removed from 3 ml of blood from each individual and peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation. PBMCs were stained with PC5 conjugated anti-CD19, PE conjugated anti-CD21 (BD Biosciences, USA) following the manufactures' instructions. AchR binding on PBMCs (AchR specified B cells) were detected by staining PBMCs with APC-conjugated AchR (from *Torpedo californica*) and APC-conjugated ovalbumin (OVA) as a control (Allman et al., 2011). AchR was purified from *Torpedo californica* electric organs (Aquatic Research Consultants, CA) according to published method (Wu et al., 2001). AchR was then dialyzed in PBS by using Spectra/Por dialysis tubing (12–14,000 molecular weight). AchR was labeled with APC Protein Labeling Kit (Invitrogen) according to the manufacturer's instructions. For detecting intracellular expression of IgG, PBMCs were fixed and permeabilized using a cytoperm/cytofix kit (BD Biosciences, USA), and then stained for FITC-conjugated anti-IgG (Biolegend) following the manufacturer's instructions. CD19 was gated to determine population of B lymphocytes. All the samples were analyzed with a BD FACS™ Calibur Flow Cytometer.

2.3. RT-PCR

The total RNA was prepared from PBMCs using Trizol Reagents (Invitrogen Life Technologies). The reverse transcription was performed using Pure Extreme first strand cDNA synthesis Kit (Fermentas Company). The PCR for CD21 genes were performed using the TransGen 2× EasyTaq SuperMix (Transgen Life Technologies) following the manufacturer's protocol. The CD21 specific sense primer is: 5′ ATTGCTGGACAGGGAGTTGCTT 3′ and the antisense primer is: 5′ TGCCCATTGAGGATGTTAG GAG 3′. The β-actin sense primer is: 5′ AGCGAGCATCCCCCAAAGTT 3′ and the antisense primer is: 5′ TTACTACTCGGAAGCACGGG 3′. The RT-PCR conditions were as follows: 94 °C for 30 min, and 34 cycles of denaturing at 94 °C for 30 s, annealing at 56.8 °C for 30 s, and extension at 72 °C for 30 s with a final extension at 72 °C for 10 min.

2.4. ELISA for serum sCD21 and anti-AchR IgG

The concentrations of sCD21 in sera of MG patients and HCs were tested following the instructions of human CD21 ELISA kits (Becton, Dickinson and Company, China), the optical density was measured at 450 nm and reference wave length was at 690 nm. All the value were read by Automatic microplate reader (Perkin Elmer company, USA), and the data was analyzed by CurveExpert 1.3 software.

The level of anti-AchR IgG in sera of MG patients was measured with method as previously described (Liu et al., 2011). Briefly, purified AchR was diluted with PH 9.6 carbonate bicarbonate buffer in a final concentration 0.5 μg/ml. 100 μg diluted AchR was coated onto each well in a 96-well microtiter plate overnight at 4 °C. 100 μl diluted serum sample (1:1000) was added and incubated at 37 °C for 90 min. Sera from healthy persons were used for background control. Each sample was duplicated. Subsequently, 100 μl HRP goat anti-human IgG (Southern Biotech, USA) in 0.01 M PBS-Tween20 (1:500, PH 7.4) were added and incubated at 37 °C for 90 min. The mixture was shaking in the dark at 37 °C min to develop color. Plates were read at 450 nm by Automatic microplate reader (Perkin Elmer company, USA), and the data was analyzed by CurveExpert 1.3 software.

2.5. Statistical analysis

All the quantitative values were described as average ± standard deviation ($\bar{X} \pm S$). The comparison of averages of two independent samples was performed by Student's *t* test. The analysis of correlation

Table 1
General information of study subjects.

Information	MG patients	Subgroup IIa	Subgroup IIb	HCs
Number of cases	20	12	8	20
Age (years)	40.40 ± 11.70	39.42 ± 12.82	41.88 ± 10.44	41.45 ± 12.21
Male:female	1:1.50	1:1.40	1:1.67	1:1.50
Disease course	15 day–10 year (mean 30 month)	3 month–10 year (mean 42 month)	15 day–2 year (mean 15 month)	–
QMG scores	17.6 ± 4.26	16.75 ± 3.77	18.88 ± 4.94	–

HCs: healthy controls.
MG: myasthenia gravis.

between two variants from two was done using Bivariate correlate test. Non-parametric two tail $p<0.05$ was taken as having significant difference. All the data were analyzed using SPSS11.7 software pack.

3. Results

3.1. MG patients and subgroups harbored elevated frequencies of AchR specified B cells

We first determined whether the frequencies of AchR specified B cells were altered significantly between MG group and HCs. The results demonstrated that the frequencies of AchR specified B cells were significantly higher in MG groups than HCs ($p<0.01$) (Fig. 1). The frequencies of AchR specified B cells in PBMCs of MG patients ranged from 0.22% to 2.78% (mean 1.90%), while the percentage of HCs ranged from 0.18% to 1.57% (mean 0.87%). In MG subgroup IIa the percentage of AchR specified B cells ranged from 0.71% to 2.93% (mean 2.05%), and in subgroup IIb it ranged from 0.22% to 3.05% (mean 1.95%), which showed no significant difference between IIa and IIb ($p>0.05$).

3.2. MG patients showed decreased frequencies of CD21$^+$ B cells, and increased frequencies of CD21$^+$AchR specified B cells in the periphery

In order to investigate the role of CD21 in MG, we measured the frequencies of CD21$^+$B cells and CD21$^+$ AchR specified B cells in the peripheral blood of MG patients (Fig. 2 A–C). The percentage in HCs ranged from 4.87% to 22.23% (mean 12.44%), while the percentage of CD21$^+$B cells in PBMCs of MG patients ranged from 3.28% to 10.96% (mean 7.84%), which showed significant decrease compared to that of HCs ($p<0.01$). The percentage of CD21$^+$B cells in PBMCs in subgroup IIa ranged from 3.28% to 10.96% (mean 7.56%), and in subgroup IIb ranged from 6.02% to 10.72% (mean 8.26%), which showed no significant difference compared to each other ($p>0.05$). We further explored the percentage of CD21$^+$ AchR specified B cells in PBMCs of MG patients and HCs. The results showed that in HCs it ranged from 0.08% to 1.26% (mean 0.53%), while in MG patients the percentage ranged from 0.21% to 2.71% (mean 1.52%), which showed significant increase compared to that of HCs ($p<0.01$). The percentage in subgroup IIa ranged from 0.5 to 2.86% (mean 1.45%), and in subgroup IIb it ranged from 0.21% to 2.71% (mean 1.63%), which showed no significant difference compared to each other ($p>0.05$).

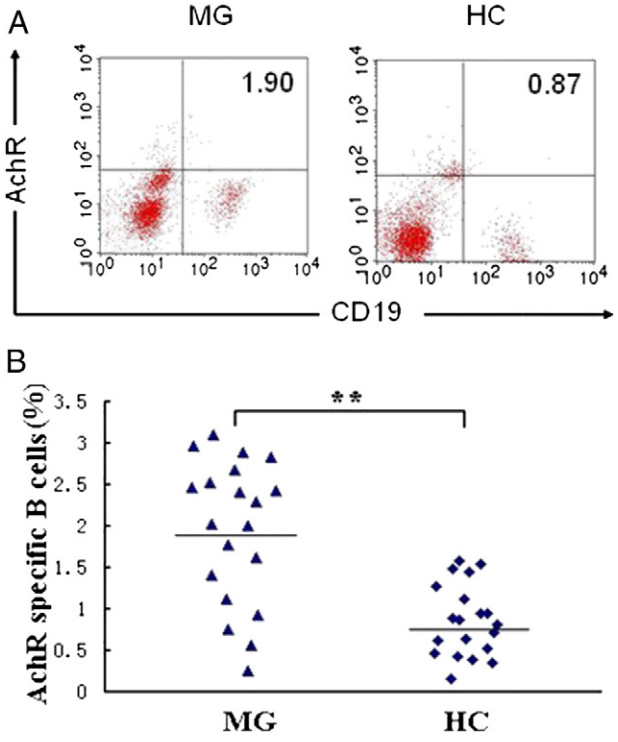

Fig. 1. Comparable frequencies of AchR specified B cells between MG patients and HCs. The peripheral frequencies of AchR specific B cells were analyzed by flow cytometry. AchR and CD19 double positive in PBMCs are reflective of AchR specified B cells. (A) Examples of FACS staining of peripheral AchR specified B cells in MG patients and HCs. (B) Representative dot plots of AchR specified B cells between MG patients and HCs (**$p<0.01$).

3.3. MG patients exhibited increased percentages of IgG$^+$ B cells and IgG$^+$ AchR specified B cells

Additionally, we detected the percentages of IgG$^+$ B cells and IgG$^+$ AchR specified B cells in PBMCs of MG patients and HCs (Fig. 3 A–D). As expected, MG patients exhibited elevated percentages of IgG$^+$ B cells (ranging from 0.21% to 1.81%, mean 1.02%) and IgG$^+$ AchR specified B cells (ranging from 0.12% to 1.83%, mean 0.69%) as compared with HCs (IgG$^+$ B cells ranging from 0.18% to 0.68%, mean 0.44%; IgG$^+$ AchR specified B cells ranging from 0.07% to 0.52%, mean 0.21%), which showed significant difference (both $p<0.01$). The percentages of IgG$^+$ B cells in subgroup IIa ranged from 0.21% to 1.81% (mean 1.09%) and in subgroup IIb ranged from 0.21% to 1.54% (mean 0.92%), which showed no significant difference between IIa and IIb ($p>0.05$). Similarly, the percentages of IgG$^+$ AchR specified B cells in subgroup IIa ranged from 0.12% to 1.83% (mean 0.71%), and in subgroup IIb ranged from 0.49% to 0.95% (mean 0.66%), which showed no significant difference between IIa and IIb either ($p>0.05$).

3.4. Expression of CD21mRNA in PBMCs

From the flow cytometry analysis, we noticed an important phenomenon that MG patients had increased frequencies of CD21$^+$ AchR specified B cells and decreased levels of CD21$^+$ B cells in the periphery. To explore the mechanism why the levels of CD21$^+$ B cells decreased in the periphery, we measured the mRNA levels of CD21 in PBMCs, predicting that the alteration of CD21 expression might be derived from the mRNA level. However, RT-PCR results of CD21mRNA level showed that the level of CD21mRNA was not significantly different between MG patients (0.548 ± 0.081) and HCs (0.541 ± 0.091), neither between the subgroup IIa (0.540 ± 0.100) nor subgroup IIb (0.581 ± 0.046) (both $p>0.05$) (Fig. 4).

3.5. Concentration of sCD21 in sera

sCD21, a secreted form of CD21, is shed from the extracellular domain of CD21 during B cell activation (Hoefer and Illges, 2009). The concentration of sCD21 in sera was measured with ELISA for both MG patients and HCs. The level of serum sCD21 in HCs ranged from 4.42 U/l to 10.39 U/l (mean 7.88 U/l). While in MG patients ranged from 2.32 U/l to 9.36 U/l (mean 5.15 U/l), in subgroup IIa ranged from 3.12 U/l to 9.36 U/l (mean 5.45 U/l), and in subgroup IIb ranged from 2.32 U/l to 6.61 U/l (mean 4.70 U/l), which all showed significant decrease compared to that of HCs (all $p<0.01$) (Fig. 5). In addition, the level between subgroup IIa and subgroup IIb did not have significant difference ($p>0.05$).

3.6. Correlation analysis between sCD21, CD21$^+$ B cells, CD21$^+$ AchR specific B cells, IgG$^+$ B cells, IgG$^+$ AchR specific B cells and serum anti-AchR IgG

We first selected 17 anti-AchR-Ab positive MG patients to conduct the Bivariate correlation test between peripheral cell population levels and anti-AchR IgG to examine whether CD21$^+$/IgG$^+$ B cells and CD21$^+$/IgG$^+$ AchR specified B cells correlated with anti-AchR IgG in MG patients and subgroups. We found that the level of CD21$^+$

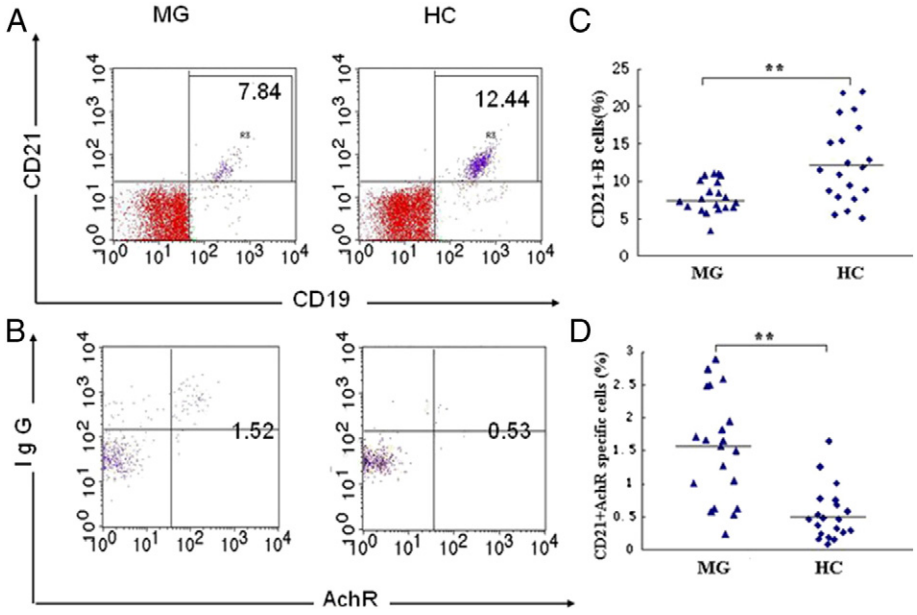

Fig. 2. Comparable frequencies of CD21$^+$ B cells and CD21$^+$ AchR specified B cells between MG patients and HCs. The peripheral frequencies of CD21$^+$ B cells and CD21$^+$ AchR specified B cells were analyzed by flow cytometry. PBMCs were first stained with CD21 and CD19, and then the double positive cells were sorted and analyzed for AchR and/or IgG expression. Data in the graph 2B are representative of quantities of CD21, CD19, and AchR triple positive cells. (A) Examples of FACS staining of CD21$^+$ B cells in MG patients and HCs. (B) Examples of FACS staining of CD21$^+$ AchR specified B cells in MG patients and HCs. (C) Representative dot plots of CD21$^+$ B cells between MG patients and HCs. (D) Representative dot plots of CD21$^+$ AchR specified B cells between MG patients and HCs (**$p<0.01$).

B cells correlated negatively with anti-AchR IgG in anti-AchR-Ab positive MG patients ($r=-0.558$, $p<0.05$). While the levels of AchR specified B cells and CD21$^+$ AchR specified B cells both correlated positively with anti-AchR IgG in anti-AchR-Ab positive MG patients ($r=0.587$, $p<0.05$; $r=0.864$, $p<0.01$. respectively). However, the levels of IgG$^+$ B cells and IgG$^+$ AchR specified B cells did not correlate significantly with anti-AchR IgG in anti-AchR-Ab positive MG patients (respectively, $r=0.239$, $r=0.167$; both $p>0.05$) (Table 2).

We also analyzed the correlation between sCD21 and anti-AchR IgG in anti-AchR-Ab positive MG patients with the same method. We found the concentration of soluble sCD21 negatively correlated with anti-AchR IgG in anti-AchR-Ab positive MG patient ($r=-0.556$, $p<0.05$) (Fig. 6).

4. Discussion

CD21, also called CR2, is located on B cell surface with three domains, an extracellular, transmembrane and cytoplasmic domain. Usually, CD21, coligated covalently with CD19 and CD81 on B cells, is responsible for sustenance of B cell survival as well as lowering threshold of B cell activation and reducing incidence of premature B

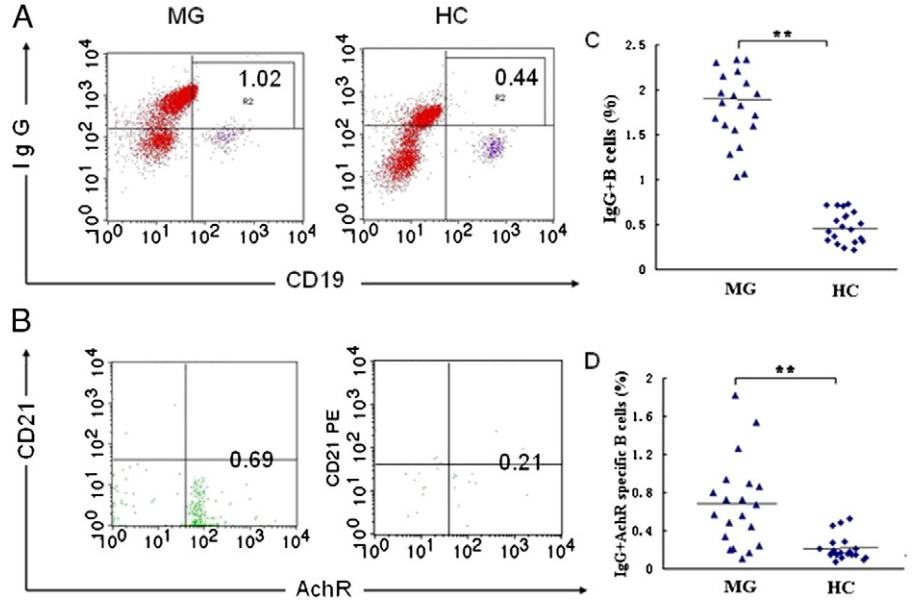

Fig. 3. Comparable frequencies of IgG$^+$ B cells and IgG$^+$ AchR specified B cells between MG patients and HCs. The peripheral frequencies of IgG$^+$ B cells and IgG$^+$ AchR specified B cells were analyzed by flow cytometry. PBMCs were first stained with CD19 and IgG, and then the double positive cells were sorted and analyzed for AchR and/or CD21 expression. Data in the graph 2B are representative of quantities of CD19, IgG and AchR triple positive cells. (A) Examples of FACS staining of IgG$^+$ B cells in MG patients and HCs. (B) Examples of FACS staining of IgG$^+$ AchR specified B cells in MG patients and HCs. (C) Representative dot plots of IgG$^+$ B cells between MG patients and HCs. (D) Representative dot plots of IgG$^+$ AchR specified B cells between MG patients and HCs (**$p<0.01$).

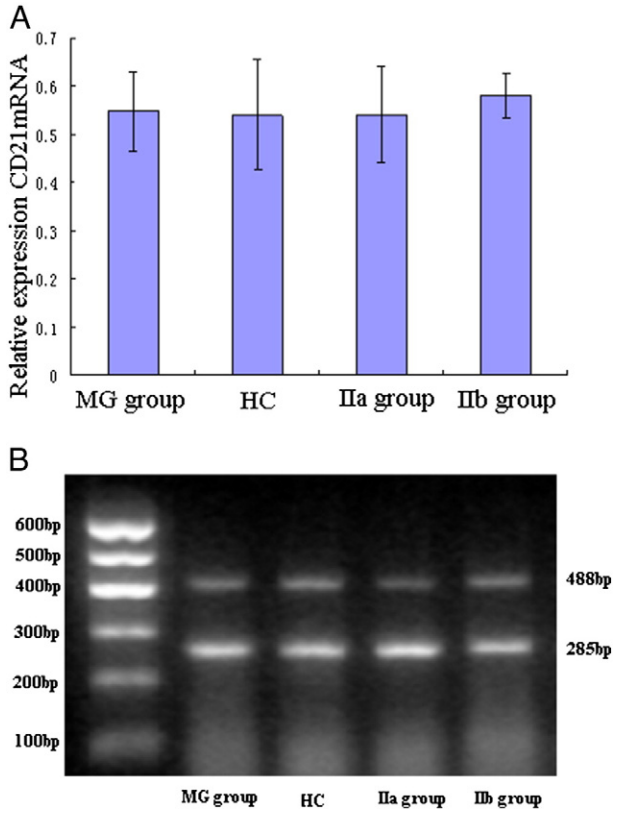

Fig. 4. mRNA level of CD21 in PBMCs of MG group, subgroup IIa and subgroup IIb, and HCs. (A) RT-PCR shows the level of CD21mRNA in PBMC of PBMCs of MG group, subgroup IIa and subgroup IIb has no significant difference with HC. (B) Representative plots for comparison of CD21mRNA of MG group, subgroup IIa and subgroup IIb with HCs ($p > 0.05$).

cell apoptosis (Molnár et al., 2008; Erdei et al., 2009; Dunkelberger and Song, 2010). Aberrant CD21 expression is involved in dysregulation of immune responses. In murine models, CD21 deficiency reduced the incidence of autoimmune disease (Boackle et al., 2004; Del Nagro et al., 2005), impaired in activation of T cell responses and secondary autoantibody responses (Del Nagro et al., 2005), and lowered the serum IgG3 levels (Boackle et al., 2004). In human autoimmune disease, CD21 expression on B cells and sCD21 titers has been observed in body fluid of patients which showed inverse relationship with disease activity. The major findings of this study are the decreased level of membrane CD21 on B cells and serum sCD21 in the periphery of gMG patients, which are consistent with previous studies that serum membrane and secreted CD21 expression were decreased in patients of multiple sclerosis (Toepfner et al., 2012), juvenile arthritis (Singh et al., 2012), systemic lupus erythematosus

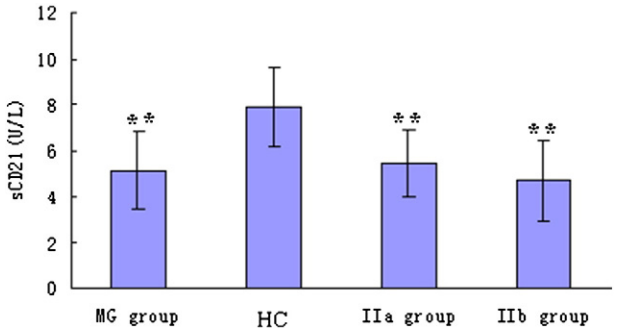

Fig. 5. Concentrations of sCD21 in sera of MG group, subgroup IIa and subgroup IIb, and HCs. Concentrations of sCD21 were analyzed by ELISA, and were showed by representative column plots (**$p < 0.01$).

Table 2
Correlation analysis between the levels of $CD21^+$ B cells, IgG^+ B cells, $CD21^+$ AchR specified B cells, IgG^+ AchR specified B cells and titers of anti-AchR IgG in anti-AchR-Ab positive MG patients.

Items	Number	Level	R
$CD21^+$ B cells	17	$7.88 \pm 2.08\%$	-0.558^*
IgG^+ B cells	17	$1.11 \pm 0.49\%$	0.239
AchR specified B cells	17	$2.01 \pm 0.78\%$	0.587^*
$CD21^+$ AchR specified B cells	17	$1.54 \pm 0.78\%$	0.864^{**}
IgG^+ AchR specified B cells	17	$0.68 \pm 0.43\%$	0.167

* $p < 0.05$.
** $p < 0.01$.

(Masilamani et al., 2004a, 2004b), rheumatoid arthritis (Erdei et al., 2009) and antiphospholipid syndrome (Singh et al., 2008). As compared with other studies of the role of CD21 in autoimmune diseases, our study found levels of $CD21^+$ B cells and serum sCD21 correlated negatively with anti-AchR IgG, which suggested that the levels of $CD21^+$ B cells and serum sCD21 might be indicative of anti-AchR IgG expression in an inverse relationship. The detailed relationship between CD21 decrease and anti-AchR IgG expression still needs exploration.

For the first time, we investigated the frequencies of AchR specified B cells and $CD21^+$ AchR specified B cells in peripheral blood of MG patients. Besides, other B cell populations involving IgG^+ B cells, IgG^+ AchR specified B cells were also detected in periphery of MG patients. In comparison with healthy controls, MG patients showed elevated frequencies of those B cell populations, suggesting CD21 along with AchR and IgG might participate in immunopathogenesis of MG. From correlation analysis, the frequencies of AchR specified B cells and $CD21^+$ AchR specified B cells had positive correlations with anti-AchR IgG. Taken into account that CD21 is crucial for B cell survival and activation (Molnár et al., 2008), we defer that increased expression of CD21 on AchR specified B cells might contribute to the initiation and progression of MG by eliciting anti-AchR IgG production.

The reason for CD21 decreases on B cells in MG needs to be explored. Previous studies suggested that membrane CD21 could be reduced in the following ways: (1) decreased transcription of CD21mRNA, (2) increased shedding of membrane CD21, (3) downregulation of CD21 mediated by ligands, such as C3b, IgE, and (4) increased terminal differentiation of CD21 (Boackle, 2005). In this study, we did not concern that the probability of CD21 decreased in the level of RNA transcription, because we did not detect any difference of mRNA level of CD21 between MG patients and healthy controls, even between the subgroup IIa and subgroup IIb. We excluded the possibility that the decrease of membrane CD21 on B cells was due to increased shedding of membrane CD21, for the reason that the levels of soluble sCD21 in serum and membrane CD21 on B cells were both decreased in periphery of MG patients. We were prone to consider that membrane CD21 was downregulated by ligation of

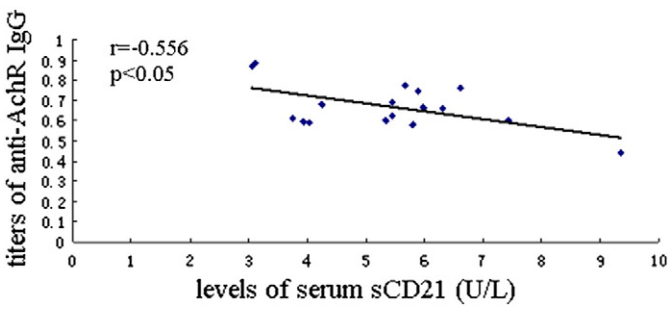

Fig. 6. Correlation analysis between serum sCD21 and anti-AchR IgG in anti-AchR-Ab positive MG patients. Serum sCD21 and anti-AchR IgG were detected with ELISA in 17 anti-AchR-Ab positive MG patients. Serum sCD21 correlated negatively with anti-AchR IgG ($r = -0.556$, $p < 0.05$).

ligands, for the level of membrane CD21 on B cells correlated negatively with the concentrations of anti-AchR IgG in the periphery of MG patients.

Serum soluble sCD21 is a shedding product from the ectodomain of membrane CD21 after B cell activation (Masilamani et al., 2003; Masilamani et al., 2004a, 2004b; Grottenthaler et al., 2006; Hoefer et al., 2008), which resembles the ligand-binding capacity of intact CD21 (Wu et al., 2001). Previous studies have shown that sCD21 can combine with CD23, C3, IgG or IgE in serum to mediate the activation of monocytes and other immunocytes (Masilamani et al., 2004a, 2004b; Grottenthaler et al., 2006; Hoefer et al., 2008). The reason why sCD21 decreased in autoimmune disease still remains unclear. Theoretically, several evidence could explain the mechanisms of sCD21 decreased in vivo of MG patients. First, following immunoactivation, more and more complement activated fragments were generated, which consumed CD21 for the subsequent function or were just in an attempt to facilitate negative feedback effect to avoid excessive immune stimulation. Second, during the course of immunoactivation, a large number of proteins were released into serum, together with protease inhibitors, such as alpha 1-protease inhibitor. CD21 shedding might be inhibited by those protease inhibitors. Third, even if CD21 was shed into serum, the sCD21 shedding product might be digested by proteolytic enzymes. In our study, the level of sCD21 correlated negatively with anti-AchR IgG, which at least indicated that the decreased level of sCD21 was due to binding of anti-AchR IgG.

In summary, the present study demonstrated that membrane CD21 on AchR specified B cell was involved in pathogenesis of MG. CD21 might be responsible for anti-AchR IgG generation of AchR specified B cells in MG patients. Further studies will be needed to monitor the changes of CD21 expression on B cell subpopulations and sCD21 in serum with disease course for MG patients. When the relationship between CD21 and the immunopathogenesis of MG becomes clear, targeting the B cell stimulating factor might be a potential therapeutic intervention of MG.

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