FOR PEOPLE LIVING WITH POMPE DISEASE,

MOBILITY CAN'T TAKE A DAY OFF

People living with late-onset Pompe disease (LOPD) face obstacles that may challenge their well-being and livelihood. A 2011 Dutch survey of LOPD patients showed[1,2]:

40% (n=32/80) stopped working due to their disease

85% required support from more than 1 caregiver to help with household tasks such as cleaning and grocery shopping

As Pompe disease progresses, it can lead to irreversible loss of mobility, respiratory function, and ability to perform daily activities, as well as premature death.[3,4] In a 2007 international study[5*]:

42% of patients with LOPD depended on a wheelchair

46% required respiratory support

Regular evaluation is recommended in patients with Pompe disease to assess for disease progression and to understand the impact on daily activities and lifestyles.[3]

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*Mean disease duration of patients studied was 11 years.

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B-CELL–ACTIVATING FACTOR IS ELEVATED IN SERUM OF PATIENTS WITH MYASTHENIA GRAVIS

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ABSTRACT: *Introduction:* Myasthenia gravis (MG) is a B-cell–mediated autoimmune disease. B-cell–activating factor (BAFF) is a major factor in B-cell development and activation. In this study we investigated serum BAFF levels in MG patients. *Methods:* We compared the serum BAFF levels of 20 MG patients with gender-matched healthy controls. We assayed serum concentrations of BAFF and anti-acetylcholine receptor antibody (AChR) titers. *Results:* Serum BAFF levels of MG patients with AChR antibodies were significantly higher than those of healthy controls. A significant positive correlation was observed between serum BAFF levels and anti-AChR antibody titers. BAFF values did not correlate with disease severity. *Conclusions:* BAFF may play a major role in the pathogenesis of MG, and it may provide a potential target for therapy in patients with MG.

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B-cell–activating factor (BAFF), also known as B-lymphocyte stimulator, is a member of the tumor necrosis factor superfamily. BAFF is a potent survival factor for B-cells and plays an essential role in the maintenance and maturation of peripheral B-cells.[1] Myeloid cells such as macrophages and neutrophils are the primary producers of soluble BAFF.[2] BAFF transgenic animals exhibit hypergammaglobulinemia, lymphoproliferation, and B-cell hyperplasia and develop autoimmune disease with manifestations that are similar to those in systemic lupus erythematosus.[3] BAFF maintains B-cell survival by modifying expression of pro- and anti-apoptotic molecules.[4]

Myasthenia gravis (MG) is a B-cell–mediated disease in which the target autoantigen is the acetylcholine receptor (AChR) at the neuromuscular junction. The cellular and molecular signals necessary for induction of human MG are not known. Because BAFF has effects on B-cells, the possibility that it plays a role in autoimmunity in patients with MG warrants further study. Several studies have demonstrated increased serum levels of BAFF in patients with MG.[5–7]

We investigated serum BAFF levels and the correlation between anti-AChR antibody titers and BAFF levels in AChR-antibody–positive MG patients at different clinical stages.

METHODS

Subjects. Twenty patients (10 women and 10 men, aged 26–79 years) with MG and 20 gender-matched healthy control subjects were enrolled in this study. The diagnosis of MG was based on the following criteria: (1) presence of fluctuating weakness of certain voluntary muscles with fatigability; (2) decremental response on repetitive nerve stimulation test; (3) positive response to an anticholinesterase injection; and (4) abnormal serum levels of anti-AChR binding or blocking antibodies. The 20 healthy control subjects consisted of volunteers with no history of any medical disease. MG patients were classified by disease stage (ocular or generalized), presence or absence of thymic abnormalities, and presence of either or both anti-AChR blocking and binding antibodies. We simultaneously assessed serum anti-AChR antibody titers to determine the relationship between serum BAFF levels and humoral immunity status. Written informed consent was obtained from all subjects. This study was approved by the ethics committee of the Jeju National University School of Medicine and performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

AChR Autoantibody Assays. AChR binding antibodies were determined by an immunoprecipitation assay using solubilized AChR complexed with ^{125}I-α-bungarotoxin. The AChR–^{125}I-α-bungarotoxin complexes to which antibodies have bound will coprecipitate with total human immunoglobulin. The radioactivity of the washed pellet is determined. An AChR binding antibody titer of $\geq$0.5 nM is considered positive. AChR blocking antibodies were assayed by a radioimmunoassay method. During incubation, AChR blocking antibodies in the sample are allowed to bind with the receptors. After incubation, ^{125}I-α-bungarotoxin is allowed to bind to the remaining binding sites on the receptors. The amount of ^{125}I-α-bungarotoxin is inversely proportional to the concentration of AChR blocking antibodies in the original sample. The percent inhibition of ^{125}I-α-bungarotoxin binding reflects

Abbreviations: AChR, acetylcholine receptor; BAFF, B-cell–activating factor; MG, myasthenia gravis

Key words: antibody; autoimmune; B-cell–activating factor; myasthenia gravis; pathogenesis; therapy

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Table 1. Clinical data and serum BAFF levels in MG patients and healthy controls.

	MG patients (n = 20)	Controls (n = 20)	P-value
Age (years)	55.4 ± 16.1	53.2 ± 15.7	0.65
Gender (men/women)	10/10	10/10	
BAFF level (pg/ml)	928.2 ± 308.1	744.3 ± 273.4	0.02
Ocular/generalized MG (n)	7/13		
Anti-cholinesterase agent only (n)	8		
Immunosuppressive agent (n)	12		
Thymoma/thymectomy (n)	2/9		

Values are presented as mean ± standard deviation. BAFF, B-cell–activating factor; MG, myasthenia gravis; n*, number of patients.*

the concentration of these antibodies in the serum sample. AChR blocking antibodies with ≥15% blocking is considered positive.

BAFF Assay. Serum BAFF levels were measured in duplicate by a quantitative sandwich enzyme immunoassay using a commercial Quantikine kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. The normal range for the BAFF assay was defined by the geometric mean BAFF levels + 2 standard deviations (SDs) in healthy controls.

Statistical Analysis. Serum BAFF levels in MG patients and healthy controls were compared using an unpaired t-test or by Mann–Whitney U-test when the data were not normally distributed. The relationship between anti-AChR antibody titers and BAFF levels was analyzed by Spearman correlation coefficients using the rank test. All statistical analyses were conducted using SPSS version 18 (SPSS, Inc., Chicago, Illinois). Values are presented as mean ± SD, and $P < 0.05$ was considered statistically significant.

RESULTS

We tested serum samples from 20 MG patients with AChR antibodies, 10 women and 10 men, aged 26–78 (mean 55.4 ± 16.1) years, and 20 gender-matched healthy controls, aged 28–76 (mean 53.2 ± 15.7) years. No significant differences were observed in age or gender between the 2 groups. Mean serum BAFF levels were significantly higher in the MG patients (928.2 ± 308.1 pg/ml) than in the healthy controls (744.3 ± 273.4 pg/ml) (P = 0.025) (Table 1). Among the 20 MG patients, 5 (25%) had BAFF levels above the normal range. A significant positive correlation was observed between serum BAFF levels and anti–AChR-antibody titers in the MG patients (R = 0.40, P = 0.04) (Fig. 1).

No significant differences were observed in serum BAFF levels between ocular (n = 7; 902.7 ± 289.1 pg/ml) and generalized (n = 13; 943.2 ± 304.3 pg/ml) MG patients (P = 0.60). BAFF values did not correlate with disease severity. Nine patients (45%) had both blocking and binding AChR antibodies, 9 (45%) had only binding antibodies, and 2 (10%) had only blocking antibodies. Serum BAFF levels showed no significant differences between MG patients with both anti-AChR binding and blocking antibodies (965.1 ± 308.5 pg/ml) and with either antibody (870.2 ± 322.4 pg/ml) (P = 0.35) (Table 2).

Among 20 MG patients with AChR antibodies, 8 were taking anti-cholinesterase only, and the others were being treated with immunosuppressive therapy at inclusion. In MG patients on immunosuppressive therapy, mean serum BAFF levels (794.9 ± 338.5 pg/ml) were significantly lower than those for patients taking anti-cholinesterase only (1,061.4 ± 217.3 pg/ml) (P = 0.02). Thymectomy had been performed in 9 patients who had thymoma and early-onset generalized MG. Serum BAFF levels in the thymectomy patients were 915.2 ± 337.1 pg/ml, whereas those without thymectomy were 941.1 ± 296.2 pg/ml, which was not statistically different (P = 0.82) (Table 3).

DISCUSSION

BAFF is important in the development and maturation of B-cells, and it plays an essential role in B-cell homeostasis and function in the periphery. There is increasing evidence that BAFF is involved

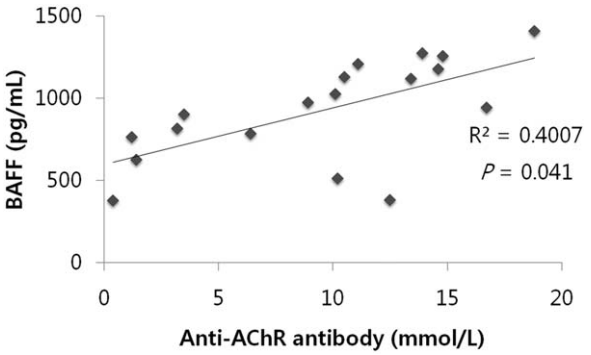

FIGURE 1. Positive correlation between B-cell–activating factor levels and anti-acetylcholine receptor antibodies in patients with myasthenia gravis.

Table 2. Serum BAFF levels in ocular and generalized MG and in MG patients with both binding and blocking anti-AChR antibodies or either antibodies.

	Ocular MG	Generalized MG	*P*-value	Both antibody	Either antibodies	*P*-value
Number	7	13		9	11	
BAFF level (pg/ml)	902.7 ± 289.1	943.2 ± 304.3	0.60	965.1 ± 308.5	870.2 ± 322	0.35

Values are presented as mean ± standard deviation. BAFF, B-cell–activating factor; MG, myasthenia gravis.

in the pathogenesis of several autoimmune diseases, including MG.[8] Increased serum levels of BAFF in MG patients have been reported in several studies.[5–7] In addition, some studies have demonstrated increased serum levels of BAFF in patients with autoimmune diseases, including systemic lupus erythematosus, Wegener granulomatosis, rheumatoid arthritis, Sjögren disease, and neuromyelitis optica.[9–13] In our study, BAFF levels were significantly higher in AChR-antibody–positive MG patients than in controls. We also demonstrated a positive correlation between circulating BAFF levels and anti–AChR-antibody titers. These results are consistent with previous studies[5–7,14] with regard to the relationship between BAFF levels and AChR antibody status and immunosuppressive treatment. In addition, we found marked variability in BAFF levels in MG patients, as reported in other studies. Unlike the study by Kim *et al.*,[5] we and others demonstrated the effect of immunosuppressive treatment on BAFF levels.[7,14] We hypothesized that BAFF would play a key role in the pathogenesis of MG; however, BAFF values did not correlate with disease severity. In MG patients, mean serum BAFF levels did not differ significantly between ocular and generalized MG. Serum BAFF levels tended to be higher in MG patients with both binding and blocking anti-AChR antibodies than in those with either antibody, but the difference was not statistically significant. These results may be attributed to the small number of subjects. Further studies with larger numbers of patients are needed to elucidate the relationship between serum BAFF levels and disease severity in MG patients.

It is known that the thymic germinal center environment provides signals that promote autoreactive B-cell survival, activation, and maturation in MG.[15] Thymic abnormalities are found in patients with autoimmune MG. The myasthenic thymus is implicated in initiating or perpetuating the disease process. Increased expression of BAFF and its receptors has been demonstrated in the thymus of patients with MG,[16,17] and most patients improved after thymectomy. However, in our study, no significant difference was observed in serum BAFF levels between MG patients with or without thymectomy. On the other hand, in MG patients being treated with immunosuppressive therapy, mean serum BAFF levels were significantly lower than those of MG patients taking anti-cholinesterase only. This finding is in agreement with recent reports on glucocorticoid-induced BAFF suppression.[18] Therefore, BAFF could be a surrogate marker for therapeutic effect with immunosuppressive agents. However, because only a small number of patients were enrolled in our study, the results must be interpreted with caution when making a generalization. Further studies are needed to clarify the significance of thymectomy and immunosuppression in relation to BAFF production.

The primary source of BAFF production is the myeloid lineage.[2] The signals that modulate BAFF expression are not fully understood. Marked variability in BAFF concentration was detected in our MG patients and could be related to BAFF gene polymorphisms.[19] There is some evidence that BAFF may also serve as a co-stimulant in T-cell responses, although this work related to animal models. Previous studies have demonstrated that production of the anti-AChR antibody was T-cell–dependent in MG. The role BAFF plays in T-cells in MG is not clear, but the BAFF interaction with T cells seems to be through BAFF receptors.[20,21] Several BAFF antagonists exist, and treatment of patients with these drugs may offer an additional approach to treating patients with MG.

Table 3. Serum BAFF levels in MG patients who underwent thymectomy and those without thymectomy and among subgroups classified by treatment modality.

	Thymectomy	No thymectomy	*P*-value	Anti-cholinesterase	Immunosuppressive agent	*P*-value
Number	9	11		8	12	
BAFF level (pg/ml)	915.2 ± 337.1	941.1 ± 296.2	0.82	1,061.4 ± 217.3	794.9 ± 338.5	0.02

Values are presented as mean ± standard deviation. BAFF, B-cell–activating factor; MG, myasthenia gravis.

The limitations of this study are the relatively small number of patients enrolled and the inclusion of only MG patients with AChR antibodies. In addition, the proportion of generalized MG patients among the enrolled patients was relatively small. One cannot necessarily apply the results of our study to patients with MG who are seronegative or positive for MuSK antibody. However, some seronegative MG patients are thought to have low titers of AChR antibodies.

In conclusion, serum BAFF levels were higher in MG patients with AChR antibodies than in healthy controls. A positive correlation was observed between serum BAFF levels and anti–AChR-antibody titers. In addition, serum BAFF levels were lower in MG patients undergoing immunosuppressive therapy than in those taking anticholinesterase only. These results suggest that BAFF is a major factor in the pathogenesis of MG. BAFF antagonists may provide new treatment options for MG patients who have failed to have a sufficient response to symptomatic treatments or standard immunosuppressive therapies.

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