IL-6 Attenuate the Efficacy of Treatment with Glucocorticoid in the Patients with Myasthenia Gravis

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Introduction

Myasthenia gravis (MG) is an autoimmune disorder generally mediated by antibodies against the acetylcholine receptors (ACh-R) of the skeletal muscles. Oral glucocorticoids (GC) are used as the first-line treatment and are still the most common agent used for long-term immunosuppression for management of MG. However, many patients have difficulties in daily activities due to insufficient improvement and adverse side effects. It has been reported that the efficacy of GC is mainly mediated by their binding to GC receptor - α (GRα), well investigated isoform. In addition to GRα, another isoform deficient in hormone binding capacity termed as GRβ has been isolated in humans. Both α and β variants are alternative splicing of pre-mRNA. It has been reported that GRβ functions as a dominant negative inhibitor of GRα transfected cells and it has been suggested that the effect is due to the formation of GRα- GRβ heterodimers. On the other hand, there has been a report denying the negative effects of GRβ is controversial, the clinical evidence demonstrated the relationship between the expression of GRβ and GC resistance has been reported.

Interleukin-6 (IL-6), originally identified as a B cell differentiation factor, has a variety of biologic functions. IL-6 induces cytokotic T cells from murine thymocytes in the presence of IL-2. In addition, IL-6 also induces the differentiation of human B lymphocyte synergistically with GC. In muscle biopsies from MG patients, IL-6 protein level was higher than in control muscle. The mechanisms underlying the increased production of IL-6 by the muscle cells after the attack by the anti-ACh-R antibodies are not clear. CD4-CD25 regulatory T (Treg) cells express the forkhead/winged helix transcription factor (Foxp3) and play important roles in the maintenance of immune tolerance. It is reported that IL-6 inhibits to induce Treg cells in periphery.

In this study, we aim to investigate the relationship between the IL-6 concentration in plasma and clinical efficacy with GC in the MG patients.

Methods

Patients
The study was approved by the ethic committees of Tokyo Medical University, and written consent was obtained from 37 MG patients (male: female = 15:22, 63.6±15.1 years old) and 6 healthy subjects (male: female = 3:3, 39.7±12.9 years old).

Isolation of PBMCs
Twenty milliliters of heparinized blood were loaded on 6 ml of Ficol-Hypaque and isolated PBMCs.

Detection of Treg cells by flowcytometry
The cells were analyzed with a FACSCanto flow cytometer using FACSDiva software. The cells were stained with PE-conjugated Foxp3, APC-conjugated CD25 and PerCP-Cy5.5 conjugated CD4 specific monoclonal antibody.

Detection of plasma IL-6 levels by ELISA
IL-6 concentrations in plasma from the MG patients were quantified by quantitative sandwich enzyme immunoassay using Quantikine® kit.

RNA extraction and quantitative real-time RT-PCR
Total RNA from PBMCs was extracted and GRα, GRβ and GAPDH mRNA expressions were determined by RT-PCR using specific Taqman probes. ΔΔCT method was adopted for the analysis of both mRNA.

MGFA post-intervention status
We evaluated the clinical status by using MGFA post-intervention status, which based on their treatment and the responsiveness for the treatment.

Statistical analysis
Statistics were carried out by Bonferroni/Dunn multiple comparison test among 3 or more different groups. The relationships between any two indices were analyzed with Pearson’s correlation test.

Results

1. IL-6 concentration in plasma from MG patients
IL-6 concentration in plasma among 37 MG patients were significantly higher than those of healthy subjects (n=6) (p=0.042). In the MG patients that were categorized in MM-2, these patients have received low-dose cholinesterase inhibitors, the IL-6 concentration in plasma were higher than those CSR and PR patients group (p=0.041)(Fig.1). We did not show the correlation between the IL-6 concentration in plasma and the frequency of CD25-Foxp3+ T cells in CD4+ T cells (Fig.2).

2. GC receptor expression on PBMCs of MG patients
We divided the patients into 2 groups based on the daily PSL dosage and GRα or GRβ mRNA levels among the MG patients groups and healthy subjects. In the MG patients who were treated with PSL (&lt;5mg/day)(n=14), GRα mRNA levels were lower than those in healthy subjects (p=0.016)(Fig.3A). There were no significant difference of GRβ mRNA levels among groups (Fig.3B).

3. Influence of IL-6 concentration in plasma on the efficacy with GC treatment in MG patients
There were no significant correlations between GRα (A) or GRβ (B) mRNA expressions in PBMCs and IL-6 concentration in plasma of MG patients (Fig.4). However, the changing rate of anti-ACh-R antibody titer was significantly correlated with IL-6 concentration in plasma of MG patients who were treated with GC for 3 months (P&lt;0.0001)(Fig.5). A. GRα mRNA B. GRβ mRNA

Conclusions

We found the GC treatment results in decreased expression of GRα mRNA expression in PBMCs, although the involvement of IL-6 concentration in plasma of MG patients was not demonstrated. The increased production of IL-6 in plasma of MG patients may attenuate the production of anti-ACh-R antibodies. The treatment to reduce IL-6 may have a significant impact on the MG patients.