Increased Complement Consumption in MuSK-Antibody-Positive Myasthenia Gravis Patients

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Key Words

Myasthenia gravis • Complement system • Antibody • Acetylcholine receptor • Muscle-specific receptor tyrosine kinase • Alternative pathway

Abstract

Objective: To investigate the activation of different complement pathways in myasthenia gravis (MG) subtypes. **Subjects and Methods:** Levels of complement breakdown products for different complement pathways were measured using ELISA in sera of acetylcholine receptor antibody (AChR-Ab)-positive (n = 21), muscle-specific receptor tyrosine kinase (MuSK)-Ab-positive (n = 23) and seronegative generalized MG patients (n = 21) and healthy controls (n = 22). Levels of factor Bb (FBb), the breakdown product of factor B, and C4d, the breakdown product of C4, were measured to evaluate the activity of the alternative and classical complement pathways, respectively. Serum iC3b levels were analyzed to assess total complement activity. The results were expressed as OD values. **Results:** MuSK-Ab-positive MG patients had a significantly higher mean concentration of serum FBb (0.638) than other MG subtypes (0.446 for AChR-Ab-positive, 0.537 for seronegative MG patients) and healthy controls (0.434) (p = 0.045). Mean serum iC3b (1.549–1.780) and C4d (0.364–0.395) levels were comparable among the groups. **Conclusion:** Our results suggest that MuSK-Ab-positive MG patients

might have a complement-activating serum factor and the alternative complement pathway might be involved in the pathogenesis of the disease. Copyright © 2011 S. Karger AG, Basel

Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by fluctuating muscle weakness and fatigue induced by antibodies directed against the acetylcholine receptor (AChR) located at the neuromuscular junction (NMJ). Complement activation induced by AChR-antibody (AChR-Ab) appears to play an important role in NMJ destruction [1, 2]. The MG patients with higher AChR-Ab levels have lower serum levels of C3 and C4, which are common and classical complement pathway factors, respectively [1]. Moreover, AChR-Ab-positive but not seronegative MG patients have reduced serum C3 concentrations as compared to healthy subjects [2], indicating overconsumption of the complement factors by AChR-Ab-induced activation of the complement system. Since most muscle-specific receptor tyrosine kinase antibody (MuSK-Ab)-positive MG patients do not display NMJ complement deposits and MuSK-Ab is predominantly of the noncomplement fixing IgG4 isotype [3, 4], involvement of the complement

system in MuSK-Ab-positive MG has been considered unlikely.

In this study, we aimed at exploring the complement pathways involved in the pathogenesis of different MG subtypes. For this purpose, levels of complement breakdown products were measured in the sera of AChR-Ab-positive, MuSK-Ab-positive and seronegative MG patients. Levels of factor Bb (FBb), the breakdown product of factor B, and C4d, the breakdown product of C4, were measured to evaluate the activity of the alternative and classical complement pathways, respectively. Serum iC3b levels were analyzed to assess the activity of both pathways, hence total complement activity.

Subjects and Methods

Patients and Sera

Sixty-five generalized MG patients (female/male: 53/12, age: 12–73 years old) were included in the study. The diagnosis of MG was based on previously defined clinical parameters [1, 2]. Patients with purely ocular muscle weakness, pregnancy, thymoma and thymectomy within 1 year before blood sampling were excluded. The included patients were divided into three groups: antibody status as AChR-Ab positive ($n = 21$), MuSK-Ab positive ($n = 23$) and seronegative ($n = 21$). Twenty-two healthy individuals of comparable age and gender were included as a control group. Neither the patients nor the controls had a systemic infection, another autoimmune disease or were under immunosuppressive treatment during blood sampling. The study protocol was approved by the Institutional Review Board. All patients and healthy controls gave written informed consent. Blood samples were collected by venipuncture and centrifuged at 3,000 rpm at 4°C for 15 min. Sera were aliquoted immediately, stored at –80°C and not thawed until assayed.

AChR-Ab and MuSK-Ab Assays

Serum AChR- and MuSK-specific antibodies were measured using a standard radioimmunoassay method with human ^{125}I -AChR or ^{125}I -MuSK antigens and using kits (DLD Diagnostika GmbH, Hamburg, Germany), according to the manufacturer's instructions. Titers of >0.6 nmol/l and >0.05 nmol/l were considered positive for AChR-specific and MuSK-specific antibodies, respectively.

Assays for the Complement Breakdown Products

Serum FBb, iC3b and C4d levels were measured using ELISA kits (Quidel, San Diego, Calif., USA) as per manufacturer's instructions. The plates were read at 405 nm for iC3b and C4d and 450 nm for FBb and the results were expressed as OD values.

Results

Serum FBb levels (mean, 0.638; range, 0.169–1.204) of MuSK-Ab-positive patients were moderately but significantly elevated as compared to AChR-Ab-positive pa-

Fig. 1. Serum FBb, iC3b and C4d levels of AChR-Ab-positive, MuSK-Ab-positive and seronegative generalized MG patients and healthy controls. Horizontal lines indicate median values for each group.

tients (mean, 0.446; range, 0.145–0.975), seronegative patients (mean, 0.537; range, 0.115–1.007) and healthy controls (mean, 0.434; range, 0.179–0.774) ($p = 0.045$ by ANOVA and Tukey's post hoc test; fig. 1). Seronegative MG patients showed trends towards displaying increased FBb levels, but without obtaining statistical significance

by post hoc analysis. Serum iC3b ranged from 1.549 to 1.780, while C4d levels from 0.364 to 0.395 and both factors were essentially comparable among all groups ($p = 0.359$ and $p = 0.896$, respectively by ANOVA) (fig. 1).

Discussion

Complement breakdown products are generated during the activation of the complement system and consequent consumption of the complement factors. Therefore, serum levels of these products are indicative of the amount of complement factor cleavage and thus the degree of complement activation [5]. Utilizing this approach in different MG types, we found increased alternative pathway activity in MuSK-Ab-positive patients. However, our results failed to show a significantly enhanced total or classical complement pathway activity in MG patients. In contrast with the previous reports [1, 2], AChR-Ab-positive MG patients did not display increased complement activity in our study. To avoid the interference of medications with the complement activity, we excluded the patients receiving immunosuppressive treatments. As a result, most of the patients inadvertently had either mild MG symptoms or were under remission during blood sampling. This factor might have led to relatively reduced complement breakdown product concentrations in all MG groups.

Some MuSK-Ab-positive patients have been shown to display NMJ complement deposits [3] and complement-

activating serum IgG1 capable of inducing membrane attack complex deposition upon binding MuSK-expressing cells [4]. Our findings confirmed these results and suggest that at least in a fraction of MuSK-Ab-associated MG, the complement system might have a pathogenic significance. The factor(s) triggering the alternative pathway activity in MuSK-Ab-positive MG patients require(s) to be identified. While serum antibodies are the foremost candidates, non-antibody factors identified in the circulation of seronegative MG patients [6] should also be considered.

MuSK-Ab-positive MG patients are known to often have a severe disease course requiring aggressive treatment. Nevertheless, they respond quite favorably to treatments such as plasma exchange and rituximab, which target the depletion of complement-fixing serum antibodies and other humoral factors [7]. These treatment methods might be exerting their beneficial effects at least partially through the reduction of increased complement activity in MuSK-Ab-positive patients.

Conclusion

Our results suggest that MuSK-Ab-positive MG patients might have a complement-activating serum factor and the alternative complement pathway might be involved in the pathogenesis of this MG subtype.

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