# Immunobiochemical Characteristics of IgG Antibodies in Myasthenia

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The abzyme activity of mAb35 and pQ1-209 was compared with that of normal rabbit IgG and serum IgG from patients with various forms of myasthenia. It was found that mAb35 and pQ1-209 and IgG from patients with myasthenia possess catalytic activity. IgG from myasthenia patients with thymomas possess creatine phosphokinase activity, which 2-fold surpassed the control.

**Key Words:** *abzymes; monoclonal antibodies; serum IgG antibodies; myasthenia*

Acetylcholine receptor (ACR) is the main target antigen in myasthenia. Anti-ACR antibodies block ACR on the postsynaptic membrane and disturb neuromuscular transmission [4,8,11]. Serum antibody pool is very heterogeneous [9,13]; 80% antibodies are directed against the main immunogenic region (67-76) of ACR α-subunits [10]. The total concentration of autoantibodies in the serum can measured by radioimmunoassay [12] and enzyme immunoassay [7], these tests confirm the diagnosis of myasthenia with up to 80% probability. The titer of autoantibodies is a reliable diagnostic test, but it cannot explain drug resistance, distribution of muscular weakness, absence of correlation between clinical manifestations of myasthenia, course of the process, *etc.* Clinical manifestations of myasthenia are described in B. M. Gekht's classification [4]. If quantitative method is sufficient for the diagnosis by Osserman's classification, polymorphism of clinical manifestations of myasthenia presented in classification by B. M. Hecht requires a special approach to investigation of serum IgG antibodies in patients with myasthenia.

A new function of IgG antibodies is now extensively studied: their capacity to catalyze various biochemical reactions in autoimmune diseases [1-3] and tumors [5]. Abzyme activity of IgG in myasthenia has never been investigated. We studied immunobiochemical characteristics of IgG antibodies in myasthenia.

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## MATERIALS AND METHODS

Conformation-dependent monoclonal antibodies (mAb35) were kindly provided by Dr. D. Lindstrem, polyclonal rabbit IgG (pQ1-209) were from Laboratory of Neuropeptide Reception (Head — Professor V. I. Tseitlin), M. M. Shemyakin and Yu. V. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences. Normal rabbit IgG served as the control. The specificity of pQ1-209 and mAb35 and the level of serum antibodies in patients with myasthenia were evaluated using enzyme immunoassay by the reaction with extracellular domain of α-subunit of human ACR expressed in *E. coli* and renatured under different conditions. The sera from patients with generalized ($n$=6) and ophthalmic ($n$=5) forms of myasthenia and patients with thymoma and myasthenia ($n$=6) treated at the Center of Neuromuscular Diseases, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, were analyzed. Control group consisted of 6 healthy volunteers. The blood was collected from the ulnar vein into a dry tube without anticoagulant and left for 2 h for clotting, after which it was centrifuged at 3000 rpm for 10 min and the serum was separated from the blood clot. Serum IgG were isolated as described previously

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**TABLE 1.** Abzyme Activity of Serum IgG Antibodies (U/mg Total Protein) in Patients with Various Forms of Myasthenia and Myasthenia with Thymoma (*M*±*m*, *n*=5-6)

| IgG abzyme activity | Control | Myasthenia | | |
|---|---|---|---|---|
| | | generalized | ophthalmic | with thymoma |
| γ-GTP | 6.8±1.6 | 6.00±0.84 | 6.2±1.3 | 6.2±2.6 |
| Alkaline phosphatase | 7.6±0.8 | 11.4±4.2 | 12.00±0.05 | 10.2±1.8 |
| CPK | 4.1±1.0 | 4.5±0.9 | 5.7±1.4 | 9.2±0.9 |

[6]. Polyclonal rabbit IgG (1 mg) were dissolved in 1 ml, and mAb35 in 0.5 ml 0.9% NaCl. The total protein content was evaluated by spectrophotometry at λ=280 nm in 1-mm cuvettes. The concentration was estimated as $OD_{280}$/1.413 ratio. Further measurements were carried out on a FP 901M semiautomatic analyzer (Labsystems) using Biocon kits. Activities of γ-glutamyl transpeptidase (γ-GTP), phosphatase, and creatine phosphokinase (CPK) were measured.

## RESULTS

pQ1-209 antibodies produced during natural polyclonal response exhibited maximum phosphatase (up to 63 U/mg) and CPK activities (61 U/mg). These activities were virtually absent in mAb35 and normal rabbit IgG. mAb35 possessed γ-GTP activity (10.5 U/mg). CPK activity of IgG from patients with myasthenia and thymoma 2-fold surpassed the control and was 1.5-fold higher than in patients with generalized myasthenia. IgG phosphatase and γ-GTP activities did not differ in different groups (Table 1). Parallel measurements of serum enzyme activities in the patients (Table 2) showed normal values.

Hence, polyclonal autoimmune response leads to the appearance of immunoglobulins with catalytic activity, which can be due to the presence of amino acid sequences characteristic of catalytic centers of enzymes, or to conformation phenomena. Interestingly, mAb35 characterized by high affinity to ACR [10], also possesses catalytic activity. This biological activity can be regarded as an additional immunobiochemical characteristic of these antibodies.

Uniqueness of the organism is determined by individual peculiarities of enzyme systems; therefore, individual manifestations of myasthenia (course of the disease, involvement of muscle groups, response to treatment, *etc.*) can be determined by these peculiarities. It was demonstrated that the appearance of proteins with immune and enzyme activities can modulate metabolism in tissues and organs involved in the pathological process [3]. This mechanism is probably responsible for polymorphism of clinical manifestations of many autoimmune diseases, including myasthenia.

IgG antibodies possessing high phosphatase activity modulate biological activity of phosphorylated proteins, in particular ACR. It can be hypothesized that IgG antibodies modifying conformation of the receptor make it more available for attack by specific antibodies followed by activation of the complement system. Conformation changes can also disturb binding of the ligand (for example, acetylcholine), which leads to disorders in neuromuscular transmission. Dynamic study of changes in the antibody γ-GTP activity can explain the development of resistance to immunosuppression, because γ-GTP participates in metabolism of glutathione, an antioxidant involved in drug detoxication. In myasthenia patients IgG antibodies possessing CPK activity can contribute to accumulation of ATP, which partially compensates dismetabolism in the muscle. On the other hand, CPK activity can provide energy for the pathological process. Our data (increased CPK activity of IgG mainly in patients with thymomas) indirectly confirm the involvement of not only synapse, but also muscle substrate in the pathological process.

**TABLE 2.** Serum Enzyme Activities (U/liter) in Patients with Different Forms of Myasthenia (*M*±*m*, *n*=5-6)

| Enzyme | Normal value | Control | Myasthenia | | |
|---|---|---|---|---|---|
| | | | generalized | ophthalmic | with thymoma |
| γ-GTP | 7-50 | 15.4±9.8 | 12.6±0.9 | 13.7±6.7 | 16.6±1.2 |
| Alkaline phosphatase | 60-300 | 115.1±11.9 | 143.8±56.5 | 139.0±30.8 | 216.1±102.2 |
| CPK | 25-200 | 65.6±13.5 | 82.8±48.6 | 53.2±24.4 | 55.7±3.5 |

The appearance of antibodies with catalytic activity in myasthenia attests to the presence of autoimmune pathology (diagnostic value) and sheds the light on their role as a specific agent modifying metabolic processes in the synapse.

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