METHODS

Differential Diagnosis of Various Forms of Myasthenia and Endocrine Ophthalmopathy by Immunoblotting

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Immunoblotting can be used for screening a population of antibodies to acetylcholine receptor subunits circulating in the blot of patients with myasthenia. *Torpedo Californica* acetylcholine receptor served as the antigen. We found that in generalized myasthenia autoantibodies bind to α_1- or α_1- and γ-subunits, while in ophthalmic form they bind only γ-subunit of acetylcholine receptor. No antibodies to any of the acetylcholine receptor subunits were detected in patients with endocrine ophthalmopathy and in healthy volunteers. This method can be used for differential diagnosis of ophthalmic myasthenia and endocrine ophthalmopathy and for predicting generalization of the pathological process in patients with myasthenia.

Key Words: *acetylcholine receptor; serum antibodies; myasthenia*

Myasthenia Gravis is an autoimmune disease associated with the production of antibodies (AB) to nicotinic acetylcholine receptors (nACR) of the muscle postsynaptic membrane, as a result of which pulse conduction from the neuron to muscle cell is impaired [9]. AB are directed to nACR determinants situated in different sites of the receptor molecule. Their pool (70-80%) consists of AB to the main immunogenic region (site 67-76) of nACR α_1-subunit extracellular domain [10]. In the majority of cases myasthenia starts from weakness of extraocular muscles, skeletal muscles can be involved (generalized myasthenia), and only in 16% cases the pathological process remains local (ophthalmic myasthenia) [2,5,6]. AB with different characteristics are detected in the sera of patients with ophthalmic and generalized myasthenia [7]. AB to ACR are detected in only 50% patients with ophthalmic myasthenia [5,6]. In ophthalmic myasthenia autoantibodies are directed against ACR fetal γ-subunit located in adults only in extraocular striated muscles [8].

Serum AB can be measured using standard kits for the diagnosis of myasthenia by enzyme and radioimmunoassays. However, these methods cannot be used for simultaneous screening of AB population.

We developed a more accurate method for differential diagnosis of conditions associated with the involvement of extraocular muscles.

MATERIALS AND METHODS

Blood sera from 10 patients with myasthenia were studied. Of these, 3 patients presented with generalized myasthenia (1 man and 2 women, 14-30 years), 3 with ophthalmic myasthenia (1 man and 2 women, 50-62 years, observed for more than 10 years), 1 patient (male) aged 63 years with ophthalmic myasthenia combined with thymoma (observed for 14 years), and 3 patients with endocrine ophthalmopathy concomitant with autoimmune thyroiditis (1 man and 2 women aged 55-70 years). Control group consisted of 3 healthy volunteers (2 men and 1 woman, 35-40 years). In addition, 3 patients with myasthenia with predominant

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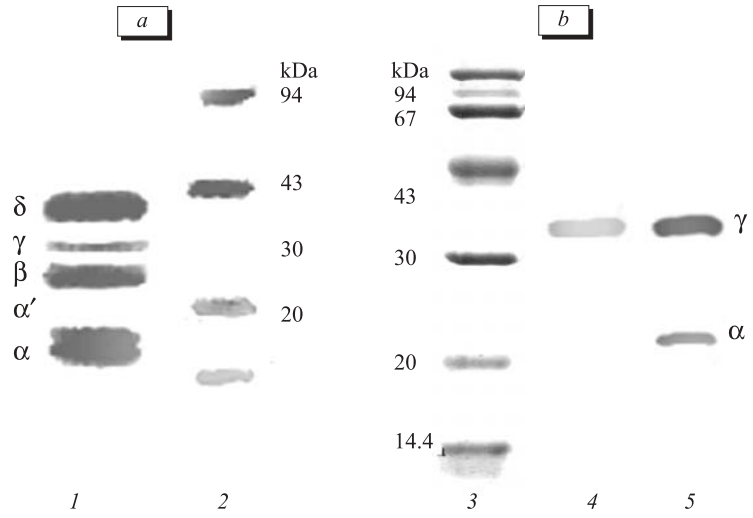

Fig. 1. Electrophoregram and immunoblotting of *Torpedo Californica* acetylcholine receptor (ACR) of blood sera from patients with ophthalmic (*a*) and generalized myasthenia (*b*). *1*) *Torpedo Californica* ACR; *2-3*) marker proteins (mol. weight in kDa is shown); *4*) ophthalmic myasthenia; *5*) generalized myasthenia.

involvement of extraocular muscles (2 men and 1 woman, 60-74 years) were examined at the debut of the disease.

Vertical PAAG electrophoresis with sodium dodecylsulfate on 1-2 mm-thick plates (113×115 and 115×175) was carried out by the standard method [3]. Two gel types were used for improving the resolving capacity of electrophoresis: concentrating (4.5%) and separating (12%), differing by the concentration of initial monomers. Ammonium persulfate (10% solution) served as the polymerization reaction initiator. *Torpedo Californica* ACR (5-15 μl) dissolved in buffer for samples and containing 3-10 μg protein was applied onto tracks. All samples were warmed in a water bath for 1-5 min before application. The optimal protocol for penetration of the samples into concentrating gel is as follows: 70-80 V voltage and 10-15 mA current. As the samples reached the borderline of separating gel, the conditions were modified: voltage 160-180 V and current 30-50 mA. Electrophoresis was carried out with cooling. Proteins were stained with coomassie blue at 50-70°C for 30 min (Fig. 1, *a*).

Specific serum AB were detected by semidry immunoblotting [1]. The conjugate-serum ratios were selected on the sera from myasthenia patients. Serum concentrations 1:49 and conjugate 1:59 were taken.

RESULTS

The study of sera from patients with generalized myasthenia showed AB binding to ACR α_1-subunit (Fig. 1, *b*). Antibodies to ACR α_1-subunit were detected in one patient (female) with local (ophthalmic) myasthenia, in addition to AB to ACR γ-subunit; this can indicate the possibility of late (period of observation more than 14 years) generalization of the pathological process. In other patients of this group AB bound to only ACR γ-subunit. Patient with local ophthalmic myasthenia concomitant with thymoma (thymomectomy was carried out 10 years before; lymphoepithelial thymoma without signs of malignant degeneration and infiltrative growth was removed) had antibodies not only to ACR γ- (as in all patients with ophthalmic myasthenia), but also to ACR δ-subunit, which can reflect heterogeneity of AB pool in patients with thymomas even in the presence of local myasthenic process.

Antibodies to nACR α-subunit were detected in all patients with oculomotor disorders at the debut of the disease; AB to γ-subunit were also present in 2 of 3 cases, while the third patient (aged 74 years) presented with AB to an extra α′ protein (rapsyn) were detected, which attested to the involvement of proteins participating in the synapse organization in the autoimmune process. Rapsyn is responsible for ACR density in the neuromuscular plexus and is located near β-subunit. The appearance of AB to it can be secondary, resulting from autoimmune attack towards ACR in seropositive myasthenia; in some patients from the seronegative group it can cause dysfunction of the neuromuscular system and myasthenia. On the other hand, the appearance of AB to rapsyn is not specific for myasthenia [4]. No AB to any of ACR subunits were detected in patients with endocrine ophthalmopathy and healthy controls.

Hence, detection of serum autoantibodies to ACR subunits by immunoblotting is an accessory method for differential diagnosis of ophthalmic myasthenia and endocrine ophthalmopathy; this method can be used for predicting generalization of the pathological process in patients with myasthenia.

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