Features of self-tolerance loss in patients with different clinical phenotypes of myasthenia


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Introduction

Nowadays, there is a worldwide steady increase in autoimmune diseases, among which myasthenia takes a special place. The disease is characterized by progressive muscular weakness; it is often accompanied by structural and functional changes in the thymus. The study of pathogenesis and treatment of myasthenia is an urgent problem due to an increase in incidence of the disease and its social significance, since the disease often occurs in young people of working age (20–40 years), and women are affected more often, with the course of the disease often being more severe compared with men (Berthi-Akinin et al., 2014; Tovazhnyanskaia & Samoylova, 2016).

The development of muscle weakness in the majority of patients is caused by disturbance of synaptic transmission in the neuromuscular synapses due to the presence of autoantibodies (AAbs) against nicotinic acetylcholine receptors (nAChR). This type of AAbs, according to various authors, is detected in 60–85% of patients examined for myasthenia (Carr et al., 2010; Phillips & Vincent, 2016).

However, autoantibodies AAbs to nAChR (nAChR AAbs) are not revealed in a large number of patients with myasthenia, and such patients are considered to be seronegative (SN) (Devic et al., 2014; Richard et al., 2013). The binding of AAbs to nAChR of muscle cells is accompanied by the activation of complement proteins and the formation of a lysis complex that violates the structural integrity of the postsynaptic membrane and prevents the signal from passing through the nerve ending (Dedaev, 2014; Tovazhnyanskaia & Samoylova, 2016). Autoimmune processes in myasthenia can affect not only the neuromuscular synapses, but other organs and tissues of the body as well (thyroid gland, adrenal gland, spleen) (Jiang et al., 2013; Lopomo & Berrih-Akinin, 2017).

The disease is often considered to be associated with the formation of thymus tumours (thymomas), and the generally accepted method of myasthenia treatment is the removal of thymus – thymectomy. However, surgical treatment for myasthenia is not always effective, and some patients have AAbs to muscle-specific tyrosine kinase (MuSK) (Huijbers et al., 2013; Koneczny et al., 2013). The binding of AAbs to MuSK of muscle cells is accompanied by the activation of complement proteins and the formation of a lysis complex that violates the structural integrity of the postsynaptic membrane and prevents the signal from passing through the nerve ending (Sprent, 2002; Kishimoto, 2002). The central carriers of AChR were expressed by thymus epithelial cells (Levinson, 2013; Makino et al., 2017; Nakamura et al., 2018). This mechanism is partially controlled by the autoimmune regulator AIRE (Berrih-Akinin, 2014). The regulator controls the representation of the nAChR peptides by the molecules of the main complex of histocompatibility (MHC) in the differentiation of T cells, which maintains immunological tolerance to the AChR (Sakaguchi, 2005; Dalakas, 2012; Ha & Richman, 2015).

The incidence of myasthenia gravis which is characterized by progressive muscular weakness on the background of structural disorders of the thymus, has increased. Myasthenia gravis is a multifactorial autoimmune disease, it has a pronounced clinical heterogeneity, and therefore the standard diagnostic and treatment protocol is not always effective. To substantiate an individual approach to the treatment of various clinical forms of myasthenia, we conducted a study of mechanisms and markers of loss of central and peripheral self-tolerance in thymus-independent myasthenia (M) and thymus-dependent myasthenia gravis with thymus hyperplasia (MH) and thymoma (MT), involving a total of 427 patients examined. In patients with different phenotypes of myasthenia, we used the methods of spectrophotometry, flow cytometry, enzyme immunoassay. In patients with MH on the background of lymphophilic thymus hyperplasia we revealed a pronounced humoral sensitization in comparison with the reference values: the concentration of C3 complement, C-reactive protein, circulating immune complexes and the initiation of an indirect autoimmune reaction – a reliable increase in autoantibodies (AAbs) to the α1 and α2 subunit of subunit of nicotinic receptors (nAChR). In M and MT groups a high similar titer of AAbs to other epitopes was revealed: DNA, β2-glycoprotein I, membranes of intestinal and stomach cells, lung, liver, kidney cells. A pronounced blast-transforming response to the presence of the mitogen PHA was revealed in the MT group. In the MT group, a decrease in the content of CD4+ CD25+ T-regulatory lymphocytes was revealed. Individual methods for correcting the loss of self-tolerance in patients with different clinical phenotypes of myasthenia were justified taking into account the use of immunosuppression, specific viral-neutralizing immunoglobulins and massive IgG immunoglobulin therapy, and the application of anti-inflammatory recombiant interleukins.
ents experience a relapse of the disease after short-term remission (Klimova et al., 2016). The role of etiological factors of immune imbalance in myasthenia may be played by bacterial and viral infections in the thymus, which change the physiological activity of cells and contribute to the development of inflammation that induces the formation of myasthenia (Barzago et al., 2016). Clinical manifestations of myasthenia are heterogeneous in terms of severity, progression, localization of neurotransmitter disturbance, and the nature of the damage to the thymus. They are characterized by a wide range of symptoms—from ptosis in local forms to severe violations of respiratory functions in generalized forms of myasthenia.

In patients with MuSK AAbs, clinical manifestations of myasthenia are non-typical in comparison with patients having nAChR AAbs, and the debut of the disease is accompanied by facial, bulbar manifestations, and weakness of the neck muscles; patients have a marked atrophy of the muscles with relative preserved work of eye muscles (Phillips & Vincent, 2016; Galassi et al., 2018).

Although complex pathogenetic therapy of myasthenia, besides thymectomy, includes a wide range of agents, such as anticholinesterase drugs, immunosuppressive and cytostatic agents and methods of extracorporeal hemocorrection, the clinical effect of treatment is not always attainable. To improve the results of therapy it is necessary to substantiate the choice of approach to individual treatment, including surgical options, using various methods of correction of the whole complex of metabolic and immunological disorders.

Autoimmune reactions in myasthenia may be the result of loss of central or peripheral self-tolerance. The mechanism of central self-tolerance loss lies in the violation of the negative selection of B lymphocytes in the bone marrow and T lymphocytes in the thymus that leads to the formation of aggressive clones of B and T lymphocytes, and AAbs (Bach, 2012). Mutations of immunoglobulin genes, the existence of cross-reactions of autoantigens with antigens of microorganisms, and sometimes just an excessive increase in the concentration of autoantigens as a result of trauma or other destructive processes leads to the awakening of sleeping autoimmune clones and the loss of peripheral self-tolerance. An important role is also played by so-called regulatory T lymphocytes (CD4+CD25+), which provide suppression of immune response to their own antigens (Dyachenko et al., 2014; Aricha, 2016; Danikowski et al., 2017). The understanding of individual immune reactions responsible for the disease development in each particular case is essential for the choice of optimal approach to myasthenia treatment (surgical intervention, immunosuppressive therapy or plasmapheresis).

The objective of our study was to substantiate an individual approach to the treatment of various clinical forms of myasthenia by study of mechanisms and markers of loss of central and peripheral self-tolerance in thymus-independent myasthenia and thymus-dependent myasthenia gravis on the background of thymus hyperplasia and thymoma.

Materials and methods

We examined 427 patients with different clinical myasthenia phenotypes. They were classified into three groups according to the structural and functional changes in the thymus and the age. The first group included 62 patients with myasthenia without thymus affection with an average age of 43 years (M). The second group included 238 patients with myasthenia against the background of thymus hyperplasia (MH) with an average age of 30 years. The third group included 127 patients with myasthenia against the background of thymoma and an average age of 45 years (MT). The components of the patients’ blood (blood cells and serum) served as materials for study.

The content of autoantibodies (AAb) against the α1 and α2 subunits of nAChR in patients with different clinical phenotypes of myasthenia was determined by the method of immunoassay using as the antigens the recombinant extracellular domains α1 (1–208) and α2 (1–208) kindly provided by Prof. S. Tzartos (Gergalova et al., 2011). The results obtained were expressed in units of optical density at a wavelength of 490 nm. The concentration of AAbs to other antigens was determined by the enzyme-linked immunoassay assay using the Eli-Viscero-Test kits (“Immunkulat”, Russia) (Polateev, 2008).

The concentration of circulating immune complexes (CIC) and the CIC constant (CIC-c) was determined by the spectrophotometric method according to the degree of precipitation in PEG6000 (Sergeeva, 1999). The levels of complement C3 and C4 components were determined by the immunoturbidimetric method (sets of FENOX Medical Solution, Belarus) (Nilsson & Nilsson, 2012).

The concentration of acute phase proteins, ceruloplasmin and haptoglobin, was determined in blood serum by spectrophotometry using the Ravin method and with rivanol, respectively (Karnyshechkiv, 2004).

The content of the C-reactive protein (CRP) was determined by agglutination in a latex-test. Quantitative determination was performed by multiple dilutions of blood serum and repeated agglutination reactions (Karnyshechkiv, 2004).

Functional activity of neutrophilic granulocytes of peripheral blood was evaluated by light microscopy according to phagocytic index (PI), phagocytic number (PA) and index of phagocytosis completion (IPC) (Maniz-Junquera et al., 2003). The oxidation-reducing activity of neutrophils was evaluated by light microscopy in NBT-test (Park et al., 1968).

The cytofluorimetric analysis of the lymphocyte population, the expression of activation markers was performed 2 hours after blood sampling, using test tubes with the K3 EDTA according to the standard protocol. In each sample, at least 10.000 cells were analyzed. Monoclonal antibodies against CD4-PE, CD25-FITC, and CD28-FITC manufactured by “Beckman Coulter”, USA, were used. For correct exclusion from the analysis zone of cells that did not meet the parameters, the necessary logical constraints were introduced into the particle distribution histogram for low-angle, side scintillation (SSC). The evaluation of expression level of surface receptors was performed at a mean intensity of fluorescence (MF). To remove erythrocytes, the sample were prepared using no-wash technology Opti-lyse C (Beckman Coulter, USA) was used. The analysis of stained cells was carried out on a flow cytometer Cyanics FC500 (Beckman Coulter, USA).

The evaluation of the functional state of peripheral blood lymphocytes was performed by their proliferative activity in cell culture without additional stimulation and under the influence of the mitogen phytohemagglutinin (PHA). The assessment of the results of lymphocytes blast transformation was performed by light microscopy (Khatov & Illina, 2009).

Concentration of IL-1β, IL-2, IL-4, in the serum was determined by solid-phase immunosassay test systems using specific monoclonal antibodies (μAbs) to IL-1β, IL-2, IL-4 (“Vector-Best”, Ukraine), sorbed on polystyrene plates, and horseradish peroxidase as an indicator enzyme.

The statistical processing of the obtained results was performed by Statistica 6.1 program. To compare average values ANOVA was used, with values P < 0.05 considered statistically significant. Experiment data is presented as (x ± SE).

Results

One of the main pathogenetic factors of autoimmune myasthenia is the presence of AAbs to nAChR (Musuda et al., 2012). Our study of such antibodies in serum of myasthenia patients revealed their high concentration in all examined groups in comparison with the control, maximum concentration of AAbs to α1 subunit of MH group (Fig. 1). By contrast, the level of antibodies to the neuronal type of the α2 subunit of nAChR was lower than the reference values of healthy people in all groups; the lowest value was observed in the MH group. Thus, in patients with myasthenia the occurrence of AAbs to muscular form of nAChR was observed in all cases that, probably, had caused the manifestation of clinical symptoms of muscle weakness. In the group of patients with myasthenia without thymus involvement and with early debut disease the AAbs content to α1 subunit nAChR was higher.

Since autoimmune processes in myasthenia are relevant not only to the muscular system, we have investigated the content of serum AAbs to a wide range of antigens. Patients of the M group showed a significant increase in the relative content of AAbs to β2-glycoprotein I 56.4 ± 12.6%, to the cytoplasmic antigens of the renal tissue 64.7 ± 0.4%, to thyroglobulin 51.5 ± 13.8%, also to DNA 30.6 ± 1.9%, AAbs to the TSH receptor 38.4 ± 6.5%, AAbs to membrane antigens of the mucous
membrane of the stomach 31.9 ± 5.3% and the small intestine 49.4 ± 2.9% of the individual average level of immunoreactivity of the examined patients (Fig. 2).

In all three groups, AAbs were detected with high frequency to the TSH receptor (in the M group with a frequency of 55.6%, in the group MН – 33.3%, in the group MT – 10.0%). In addition, 55.6% of M group patients showed AAbs to DNA, TSH receptor, and membrane antigens of hepatocyte mitochondria; 44.4% of patients had AAbs to the membrane antigens of the mucous membrane of stomach and small intestine; 22.3% – to membrane antigens of the lung tissue and myocardial cells. There was an increase in AAbs to the S100 protein, cytoplasmic liver antigens, and cytoplasmic pulmonary antigens.

Patients of the MH group showed a significant increase in AAbs to TSH receptor 84.7 ± 7.9%, and to thyroglobulin 119.6 ± 36.4%, as well as increased AAbs to mitochondria of hepatocytes 25.9 ± 0.2%, to β2-glycoprotein I – 30.6 ± 0.3%, to the protein of the astrocyte intermediate filament – 34.3 ± 0.3%, and to insulin – 29.6 ± 0.2% (Fig. 3).

The study of the relative content of AAbs (as a percentage of the individual average) showed that in the MT group the content of AAbs to DNA was 34.9 ± 2.4%, to the membrane antigens of the gastric mucosal cells – 35.5 ± 4.8%. The concentration of AAbs to β2-glycoprotein I was two times as much as that of control and amounted to 49.4 ± 11.4%, to cytoplasmic antigens of the pulmonary tissue – 43.3 ± 9.8%, to thyroglobulin – 41.4 ± 2.6%, to the TSH receptor – 35.0 ± 5.1%, to hepatocyte mitochondria – 27.4 ± 1.8% (Fig. 4).

In 40% patients of the MT group, AAbs to DNA were detected, in 30% – to membrane antigens of the gastric mucosal cells, and in 20% – to membrane antigens of the mucous membranes of the small intestine. Besides, in this group AAbs were also found to other antigens, such as membrane antigens of hepatocyte mitochondria, protein of astrocyte intermediate filament, membrane and cytoplasmic antigens of the pulmonary tissue, and to specific protein of axon myelin sheaths.

Thus, in all groups of patients with myasthenia we observed autoimmune reaction, the specificity of which was somewhat different between the groups. In patients with thymus hyperplasia the reaction was mostly directed against the muscle α1 subunit of nAChR; in other groups AAbs were found against a wide range of antigens, which can be considered as a potential factor of disorders in the respective organs and systems.

One of the mechanisms of pathogenic action of AAbs is their participation in the formation of circulating immune complexes (CIC), which, on the one hand, are functioning as a form of elimination of antibodies from the bloodstream, and on the other hand, – as an effective means of activation of immune cells. Concentration of CIC was elevated in all studied groups, (in the M group with a frequency of 55.6%, in the group MH – 33.3%, in the group MT – 10.0%). In addition, 55.6% of M group patients showed AAbs to DNA, TSH receptor, and membrane antigens of hepatocyte mitochondria; 44.4% of patients had AAbs to the membrane antigens of the mucous membrane of stomach and small intestine; 22.3% – to membrane antigens of the lung tissue and myocardial cells. There was an increase in AAbs to the S100 protein, cytoplasmic liver antigens, and cytoplasmic pulmonary antigens.
Phagocytic activity of neutrophilic granulocytes with thymic hyperplasia (MH). NBT-test were higher, but the differences in the groups were insignificant at the expense of the AFO. The oxidative reactions in the stimulated by zymosan various degrees of increase in oxidative reactions were revealed at the presence of CIC, but they were less responsive to stimulation.

Disorders were characterized by inadequate digestibility of phages, and neutrophils. The study of oxygen-dependent processes of phagocytosis in the NBT-test revealed the intensification of spontaneous reactions of oxidative metabolism in neutrophils by active forms of oxygen (AFO) in all patients with different clinical phenotypes of myasthenia; it was proved by elevated values of the spontaneous NBT-test in all groups (Table 2).

However, the stimulated NBT test indicated a decrease in the functional reserve of the oxygen mechanism of bactericidal effects of phagocytes, as evidenced by the decreased values of the stimulation index, the most pronounced in the MH group. It characterizes the high degree of activation of intracellular NADP-H-oxidase activity. Thus, neutrophils of patients with myasthenia were in pre-activated condition possibly due to the presence of CIC; but they were less responsive to stimulation.

The lowest index of stimulation was found in the group of patients with thymic hyperplasia (MH).

Consequently, in all patients with different forms of myasthenia the various degrees of increase in oxidative reactions were revealed at the expense of the AFO. The oxidative reactions in the stimulated by zymosan NBT-test were higher, but the differences in the groups were insignificant. Alongside this, the oxygen-independent phagocytosis index (PI) of neutrophils in myasthenia patients was also lowered in comparison with the reference values (Table 3); the most pronounced decrease was recorded in the MH group.

<table>
<thead>
<tr>
<th>Indicators of phagocytic activity</th>
<th>Reference values</th>
<th>Study groups (x ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M, n = 62</td>
<td>MH, n = 238</td>
<td>MT, n = 127</td>
</tr>
<tr>
<td>Phagocytic index, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phagocytic number</td>
<td>85.0 ± 5.1</td>
<td>82.7 ± 7.9*</td>
</tr>
<tr>
<td></td>
<td>74.0 ± 6.4*</td>
<td>81.7 ± 8.5*</td>
</tr>
<tr>
<td>Phagocytic number</td>
<td>3.20 ± 0.21</td>
<td>3.33 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>2.85 ± 0.60</td>
<td>3.51 ± 0.90</td>
</tr>
<tr>
<td>Index of completeness of phagocytosis</td>
<td>1.85 ± 0.12</td>
<td>1.14 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>1.06 ± 0.08</td>
<td>1.03 ± 0.19</td>
</tr>
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</table>

| Note: see Table 1. |

Fig. 5. Concentration of the C3 (a) and C4 (b) component of complement in patients with different clinical phenotypes of myasthenia gravis: M – myasthenia without thymus affection (n = 44); MH – myasthenia with thymus hyperplasia (n = 95); MT – myasthenia with thymoma (n = 67); P < 0.05 in comparison with the control

An increase in haptoglobin was observed in patients of the MT group and, to a lesser extent, the M group; the level of ceruloplasmin was even slightly lower compared to the control. The levels of C3 component in the M group did not much differ from the control values (Fig. 5a). In the MT group, the average concentration of the C3 component was also significantly higher than in control.

The concentration of the C4 component that mediates the antibody-dependent complement activation pathway was decreased in the M and MT groups (Fig. 5b).

CRP is one of the key components of humoral natural immunity that provides a connection between congenital and adaptive immune responses. Its increase in all groups of patients is consistent with high levels of AAbs in blood serum of these patients. The maximum values of CRP and antibodies to muscle nAChR were found in the MH group. Thus, the main proinflammatory factor in myasthenia was CRP, the level of which was maximally elevated in the MH group (Table 4); so it is the MH group in which the most aggressive autoimmune reaction against muscle nAChR can be expected (Tzartos & Lindstrom, 1980; Bach, 2012).

Production of AAbs depends on the activity of cells of adaptive immunity – T and B lymphocytes. In humans, the cells most available for study are peripheral blood lymphocytes, most of which belong to T
lymphocytes. We determined the proliferative activity of peripheral blood lymphocytes in patients with myasthenia in a spontaneous and PHA stimulated reaction of blast transformation, the results of which are presented in Table 5. The data show a high level of proliferative activity of peripheral blood lymphocytes on 48-hour cultivation (without mitogenic stimulation) in patients of groups M and MH. In patients of the MT group spontaneous proliferative activity did not differ from control. 

Stimulation with PHA resulted in a strong proliferative response in the MT group, a marked response in the M group, but a very weak reaction was in the MH group.

**Table 5**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Reference values</th>
<th>Study groups (x ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 25</td>
<td>M, n = 62</td>
<td>MH, n = 238</td>
</tr>
<tr>
<td>Number of blasts cells without mitogen stimulation, %</td>
<td>11.0 ± 2.5</td>
<td>22.0 ± 9.8</td>
</tr>
<tr>
<td>Number of blasts cells stimulated by mitogen PHA, %</td>
<td>45.5 ± 4.5</td>
<td>53.0 ± 7.0</td>
</tr>
<tr>
<td>Index of stimulation</td>
<td>2.60 ± 1.30</td>
<td>1.40 ± 0.61</td>
</tr>
</tbody>
</table>

Note: see Table 1.

Thus, myasthenia without thymus damage (M) was accompanied by a preactivated condition of peripheral blood T lymphocytes that retained the ability to be activated by mitogen. In patients with thymus hyperplasia (MH), T lymphocytes were also in a preactivated condition, but they were significantly less responsive to mitogenic stimulation. In patients with thymoma (MT), T lymphocytes were not preactivated, but they were potentially responding to PHA activation.

Different mechanisms of self-tolerance loss can concern processes that occur both in the central organ of immunity, the thymus, and in the peripheral immune system (Sprent & Kishimoto, 2002; Pevzner et al., 2012). An important role in supporting self-tolerance belongs to regulatory T lymphocytes. In all examined patients, a significant decrease in the expression of markers of regulatory T cells CD4+ CD28+ against the moderate reduction of total CD4+ CD25+ T lymphocytes background was found.

The maximum decrease in CD4+ CD25+ co-stimulation markers compared with control was found in the group of patients with myasthenia without morphofunctional changes in the thymus (M) – 38.0 ± 1.6% versus 62.1 ± 5.8% (Fig. 6a). In the MH group, the reduction in CD4+ CD25+ markers was less pronounced – 46.7 ± 2.1%; in the MT group, the level of these markers was higher – 52.0 ± 4.4%.

The maximum reduction in the number of Treg CD4+ CD25+ cells was found in all groups of patients with myasthenia on the background of thymus hyperplasia (MH) – 0.6% vs. 3.2% in the control. In other groups, the number of regulatory cells was higher than the MH group, but still lower than in the control: 1.9% and 2.1% respectively in the MH group.

Table 6

The cytokine level in patients with different clinical phenotypes of myasthenia (x ± SE)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Control, n = 25</th>
<th>Study groups (x ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, pg/ml</td>
<td>1.60 ± 0.30</td>
<td>1.10 ± 0.60</td>
</tr>
<tr>
<td>IL-2, pg/ml</td>
<td>2.70 ± 0.40</td>
<td>6.60 ± 5.21*</td>
</tr>
<tr>
<td>IL-4, mmol/L</td>
<td>7.00 ± 22.9</td>
<td>337.3 ± 59.4</td>
</tr>
</tbody>
</table>

Note: see Table 1.
Increase in the level of CRP is acting as opsonin through stimulation of reactions of precipitation, agglutination, phagocytosis and complement fixation.

The activity of T-regulatory CD4+ CD25+ affects the functional activity by initiating the reacti-

ators of T and B lymphocyte proliferation. Binding of C-reactive protein to the nAChR triggers the complement system (Kusner et al., 2013). It should also be noted that in the MH group contributes to the cytotoxicity of autoantibodies and is proba-

bly due to the high concentration of CIC that activates the components of the complement system (Kusner et al., 2013). It should also be noted that in the MH group the minimal level of regulatory CD4+ CD25+ T lymphocytes is determined. In these patients the most pronounced manifestation of myasthenia symptoms can be predicted, but damage to other organs and tissues can be moderate.

In patients with thymoma (MT) the cells of natural immunity (neutrophils) and adaptive immunity (T lymphocytes) are in the state of maximum activation and do not respond to additional stimulation. The humoral immune response is directed mainly against muscle nAChR (Nakamura et al., 2018). In the blood of these patients, the maximum increase in the level of CRP is acting as opsonin through stimulation of phagocytosis and cells of the monocyte-macrophage system, which causes the general state of inflammation. Proteolytic products of CRP are regulators of T and B lymphocyte proliferation. Binding of C-reactive protein to T lymphocytes affects its functional activity by initiating the reactions of precipitation, agglutination, phagocytosis and complement fixation. The elevated level of the C3 component of the complement observed in this group contributes to the cytotoxicity of autoantibodies and is proba-

bly due to the high concentration of CIC that activates the components of the complement system (Kusner et al., 2013). It should also be noted that in the MH group the minimal level of regulatory CD4+ CD25+ T lymphocytes was found that may suppress other cells in inflammatory processes. In these patients more general autoimmune reaction with lesions of various organs and tissues can be expected.

In patients with myasthenia, without damage to the thymus (M), the cells of natural immunity become activated less than in two previous groups, and the level of CRP is also lower, although substantially higher compa-

red to the control. The level and specificity of AAbs and the number of immune complexes are similar to those in the MT group.

Conclusion

Summarizing the data obtained, it is possible to characterize the state of immune reactivity of various groups of patients with myasthenia as follows. Patients with thymus hyperplasia (MH) can be recommended for plasmapheresis to remove autoantibodies and immune complexes, as well as anti-inflammatory and immunosuppressive therapy. Most likely, in patients with thymoma (MT group), the development of myasthenia is provoked by a breakdown of peripheral tolerance (activation of “sleeping” autoimmune B-lymphocyte clones) as a result of infections or the appearance of tumour antigens. Probably, in this case thymectomy is justified, as it reduces the concentration of tumour auto-

antigens. In patients with myasthenia, without damage to the thymus (M) the lymphocytes are in the activated state, but they are able to respond to stimulation, that is, there is an active immune reaction. In these patients, activation of the adaptive immune system, including both B and T lymphocytes, is evident. Obtaining new knowledge about the mechanisms of self-tolerance loss will justify the choice of approa-

ches to the treatment for various clinical myasthenia phenotypes, including thymectomy and specific types of treatment (anti-inflammatory therapy, hormone therapy, plasmapheresis, immunosuppression).

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