Vaccinations in autoimmune myasthenia gravis

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Chapter 1

General introduction

MYASTHENIA GRAVIS

Myasthenia gravis (MG) is an acquired autoimmune disease of the neuromuscular junction. The disease is characterized by fluctuating weakness and fatigability of the skeletal muscles [1, 2]. The pattern and severity of clinical symptoms can vary widely between patients and the distribution of muscle weakness can help to recognize a subtype, which can be related to the type of antibody that is present [3]. The majority of MG patients have acetylcholine receptor (AChR) antibodies. These were already described in 1973 in rabbits and in 1975 in humans [4, 5]. Other antibodies, are found less frequently and are directed to muscle-specific kinase (MuSK) in MuSK MG or to voltage-gated calcium channels (VGCC) in the Lambert-Eaton myasthenic syndrome (LEMS) [6, 7]. In rare cases also antibodies to Lrp4, or agrin have been described [8-10].

The initial trigger for developing these autoantibodies is unknown, but both T- and B-cells have to be involved in this process. There is evidence that the thymus has a crucial role in AChR MG, as many patients have either thymic lymphoid hyperplasia or a thymic tumor [3, 11]. In healthy individuals the thymus will start to show atrophy from early adulthood. In the hyperplastic thymus often lymphocytic infiltrates and germinal centers are found, and these are thought to play a role in the initiation or continuation of the immune response against the AChR. AChR expression can be activated in thymic epithelial cells through cytokine and receptor signaling, potentially triggered by a virus. However, no specific virus has been identified so far [1]. Autoreactive T cells, specific for AChR, escape the normal intrathymic surveillance and are exported to the periphery where they stimulate B cells to produce antibodies. Differences in autoantibody pattern, HLA associations, thymic pathological changes, cytokine intrathymic pattern, and T-cell subsets and clones all point to differences in induction mechanisms for early-onset, late-onset, and thymoma-associated myasthenia gravis [1]. A frequently used therapy is a thymectomy in patients with thymic hyperplasia or a thymic tumor, which has a favorable effect on the disease course and provides additional evidence that abnormal thymic function contributes to the development of MG [12].

ROLE OF AUTOANTIBODIES

Next, a closer look will be taken at the pathophysiological effect of the two most frequently found antibodies, i.e., AChR and MuSK antibodies. Serum antibodies to the AChR are usually of the IgG1 or IgG3 subclass. These antibodies can cross-link because they are bivalent and they can activate serum complement to cause complement-dependent damage to the neuromuscular junction (NMJ) [13, 14]. The latter is the most important mechanism in most patients and results in morphologic damage to the NMJ with loss of AChRs [13]. This damage to the normally highly folded NMJ postsynaptic muscle membrane results in a reduction in the number of voltage-gated sodium channels, increasing the threshold for activation of the

action potential and further impairing the efficacy of signal transmission [2, 13, 14]. Furthermore, accelerated internalization of the AChRs induced by polyvalent antibody cross-linking further reduces the AChR numbers. Direct blocking of AChR function by a variety of possible mechanisms is rarely a major mechanism [2].

Serum antibodies against MuSK are predominantly of the IgG4 subclass. Antibodies of the IgG4 subclass do not activate complement, and are considered to be monovalent for binding to MuSK, as IgG4 antibodies can “exchange” arms with other IgG4 antibodies. Thus, complement activation and antigenic modulation are not thought to play a major role in the pathogenesis of MuSK MG (compared with AChR MG). IgG4 MuSK antibodies block the agrin-induced binding of LRP4 to MuSK, which activates multiple signaling pathways that lead to aggregation of AChR and transition from the plaque-to-pretzel form of the neuromuscular synapse [14, 15]. Thus, in MuSK MG the disease mechanism leading to clinical weakness is clearly different from that in AChR MG.

IMMUNOSUPPRESSIVE MEDICATION

A large part of the patients needs long-term immunosuppressive medication, because symptomatic treatment with cholinesterase inhibitors (such as mestinon) is insufficient. Even despite immunosuppressive medication, in 10-15% of the patients full control of the disease is not achieved. This is possibly associated with severe side-effects of the medication [1].

The most frequently used medication is azathioprine combined with prednisolone, followed by mycophenolic mofetil and cyclosporine. The latter two are also frequently combined with prednisolone and are also used to reduce the dosage of prednisolone. Less frequently used medication is rituximab and eculizumab, which is mainly used in patients with more severe myasthenia gravis. In this thesis we mainly describe patients with mild to moderate and stable disease, therefore rituximab and eculizumab will not be further discussed here.

Prednisolone or prednisone is a corticosteroid that has a broad suppressing effect on the immune system. For patients who need to take long-term corticosteroids, specific precautions should be taken to reduce the risks of glucose intolerance, gaining excess bodyweight, hypertension, and development of osteoporosis.

Azathioprine is a purine antagonist, which suppresses the increase and proliferation of B- and T-lymphocytes and damages DNA by uptake of thiopurine. The most encountered side-effects of azathioprine are leucopenia and hepatotoxic effects, mainly during the first months of treatment [1]. Mycophenolic mofetil (MMF) is a second-line medicine. MMF is a prodrug that after conversion blocks purine synthesis and interferes with B-cell and T-cell proliferation. For MMF side-effects are rare, with mild headache, nausea, and diarrhea as the most commonly reported.

Ciclosporine and methotrexate seem to be as effective as azathioprine. Ciclosporine suppresses specifically (reversibly), the proliferation of T-cells, leaving phagocytic cells unaffected. Patients should be monitored for potential side-effects, especially nephrotoxic effects and hypertension [1].

In this thesis we often compare within the above mentioned treatment groups patients with and without immunosuppressive medication and those with and without a thymectomy in the past. This in order to find or exclude an effect of a treatment.

PRESENCE OF ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES AND CLINICAL ONSET

As described above, despite that the pathogenic AChR antibodies are already described decades ago, it is still unknown what the initial trigger for making these antibodies is in individual patients.

In chapter 2 of this thesis, we describe a case report of presence of anti-acetylcholine receptor antibodies 2 years before clinical onset. In this case, onset of clinical symptoms was during pregnancy, which could be considered as a immunological event. Pregnancy is a well-known trigger for the clinical onset of MG, and there is a considerable risk for clinical onset during the post-partum period [16]. We studied the anti-AChR levels before onset of clinical symptoms and during treatment in the years thereafter (Chapter 2).

Evolution of the symptoms in individual patients has a variable pattern and, because the disease can be difficult to recognize, the diagnosis can be delayed until the second year after symptom onset [17]. It is unknown how long the presence of anti-acetylcholine receptor antibodies may precede clinical symptoms. Furthermore, titers of anti-AChR antibodies as such are not a reliable biomarker for the clinical severity or the clinical change at group level, but can show a reasonably good correlation in one and the same patient [18]. Prospective monitoring of the onset of anti-AChR antibodies in a group of individuals at risk for MG would be helpful, but is not feasible due to the low incidence of the disease.

A PATIENT REPORTED OUTCOME MEASURE FOR QUALITY OF LIFE

Optimal treatment aiming to achieve mild disease manifestations or remission often requires the use of immunosuppressive medication [1]. Despite treatment, patients can experience restrictions in their daily activities and health related quality of life (HRQOL) [19]. This can be due to side effects of medication or to the disease itself. The impact of the disease on quality of life is best reported directly by the patient through Health-Related Patient Outcomes (HR-PROs) [19]. PROs are measurements

of any aspect of a patient's health status that are evaluated from the patient's perspective without interpretation of the response by a clinician or anyone else [20]. Nowadays, the focus in the clinical setting is mainly on the effect of treatment in terms of clinical symptoms. Previously, outcome measures like the MG composite (MGC) and the Quantitative Myasthenia Gravis (QMG) score have been used. These outcome measures do not assess health-related quality of life. The 15-item myasthenia gravis quality of life scale (MG-QOL15) was constructed to measure the patient's perceived HRQOL, which covers broad domains like physical, social, and psychological well-being [21, 22]. In order to use the MG-QOL15 as an outcome measure in clinical trials and standard care, it must be validated. At the moment, the MG-QOL15 has been validated in several languages [21, 23-26]. In preparation of the tetanus and influenza vaccination trials, we translated and validated it into Dutch and evaluated its measurement properties in terms of test-retest reliability and construct validity (Chapter 3).

TETANUS AND INFLUENZA VACCINATION

In case of vaccinations, which can also be considered as an immunological event, little was known in myasthenia gravis on the efficacy of vaccination and its possible effect on disease activity. This is described in the chapters 4-6.

Patients with an autoimmune disorder are believed to be more prone to infection, due to their immunosuppressive therapy or due to the immune abnormalities associated with their disease [27, 28]. In myasthenia gravis, an infection has been associated with an aggravation of the symptoms, sometimes resulting in a myasthenic crisis. Specific data on infection rates in myasthenic patients do not exist [29]. For some of these infections vaccines are available and some of them, such as the annual influenza vaccination, are recommended for patients with an autoimmune disease as MG. However, an adequate immune response to vaccination could be hampered by the dysregulation of the immune system which is evident from the development of autoimmunity or by the effect of the immunosuppressive medication on the immune system. Little is known about safety and effectiveness of vaccination in myasthenic patients and this remains a matter of debate. For the clinician, it is also important to be able to inform their patients about the risks and benefits of a vaccination. Also, because people travel more to areas for which prophylactic vaccinations are recommended.

In this thesis we therefore describe two randomized clinical trials investigating the effect of two vaccinations. The first trial investigated tetanus revaccination. We choose tetanus because it a frequently used vaccine with a well-known safety profile and antibody response in healthy individuals as well as in immunocompromised individuals with HIV or after stem cell transplantation [30, 31]. Furthermore, knowledge of tetanus revaccination in myasthenia gravis is also practical for both clinicians as patients, because patients that attend the emergency department often

need a tetanus revaccination because of the trauma they suffered (Chapter 4). The second vaccine we describe in this thesis is the annual influenza vaccination. As already mentioned above, the annual influenza vaccination is recommended for all patients with MG. No specific guidelines regarding (influenza) vaccinations in patients with MG exist, but a small number of observational studies suggest that influenza vaccination is safe [32, 33] and recently a randomized controlled trial confirmed this by the finding that influenza vaccination has no influence on the AChR antibody titre [34].

In our personal experience and as earlier described [32], many patients express concern that influenza vaccination may lead to an exacerbation and a substantial number declines vaccination each year based on this concern. This is unfortunate, as seasonal vaccination against influenza is highly effective in reducing laboratory-confirmed influenza illness, hospital admissions and risk of death, especially in elderly and frail patients [35]. This is relevant, as this age group has currently the highest incidence of autoimmune MG [36]. Therefore, we performed a double-blind, placebo-controlled trial to investigate the efficacy and safety of the seasonal (2016/2017) influenza vaccine in patients with AChR MG, with and without immunosuppressive medication (Chapter 5).

THE EFFECT OF A VACCINATION ON THE T- AND B-CELLS COMPARTMENT

Performing the above described tetanus study gave the opportunity to also investigate the cellular response to tetanus revaccination. The humoral response to tetanus is T cell dependent and the immunosuppressive medication has a general suppressing effect, also on the T cell compartment. Therefore, we investigated the *in vitro* tetanus-specific T cell responsiveness in the same MG cohort pre and post revaccination, with a focus on the effect of immunosuppressive medication and the influence of a preceding thymectomy. Furthermore, we investigated a broad spectrum of (functional) subsets in the B- and T cell compartments pre and post vaccination (Chapter 6)

The results and conclusions of these thesis are summarized and discussed in Chapter 7.

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