We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Structure-Based Approaches to Antigen-Specific Therapy of Myasthenia Gravis

Jiang Xu, Kaori Noridomi and Lin Chen

Abstract

A majority of Myasthenia Gravis (MG) cases (~85%) are caused by pathological autoimmune antibodies to muscle nicotinic acetylcholine receptors (nAChRs). An attractive approach to treating MG is therefore blocking the binding of autoimmune antibodies to nAChR, or removing specifically nAChR antibodies, or selectively inhibiting and eliminating nAChR-specific B cells. This chapter will review high-resolution structural studies of muscle nAChR and its complexes with antibodies derived from experimental autoimmune Myasthenia Gravis (EAMG). Based on these structural analyses, various strategies, including using small molecules to block the binding of MG autoimmune antibodies, and engineered chimeric nAChR antigen to specifically target and eliminate B cells that produce nAChR-specific antibodies, will be discussed.

Keywords: crystal structure, nicotinic acetylcholine receptor, antigen-specific therapy, Myasthenia Gravis, autoimmune antibodies, chimeric nAChR antigen, nAChR-specific B cells

1. Introduction

Myasthenia Gravis (MG) is an autoimmune disease that afflicts a significant human population. MG patients suffer from a variable degree of skeletal muscle weakness. The symptoms range from mere lack of muscle strength to life-threatening respiratory failure. MG is a chronic disease that can last many years and negatively impact the quality of living and life expectancy of afflicted individuals. Although MG rate is reported to be 7–20 out 100,000 [1] and the diagnosed MG cases are increasing, probably due to increased awareness of this debilitating disease, the aging population and other intrinsic and extrinsic factors that disturb the human immune system [1].

The majority of MG cases (~85%) are caused by pathological autoantibodies to muscle nicotinic acetylcholine receptors (nAChRs), a ligand-gated ion channel that mediates rapid signal communication between spinal motor neurons and the muscle cells. Autoantibodies against other neuromuscular junction (NMJ) proteins, including muscle-specific kinase (MuSK) and lipoprotein-related protein 4 (LRP4), can also cause muscle weakness in a small fraction of patient [2, 3]. The heterogeneous nature of MG autoantibody presents a challenge to both diagnosis and treatment of the disease.

Current treatment regimens for MG include anticholinesterase inhibitors, thymectomy, immunosuppressants, plasmapheresis, or intravenous immunoglobulins [4].

Most MG patients respond favorably to these treatment options to achieve effective symptom relief, and in some cases even clinical remission. Cholinesterase inhibiting drugs can temporarily enhance neuromuscular transmission by delaying the breakdown of acetylcholine (ACh) to compensate for the loss of NMJ nAChRs, but this treatment option only works in a fraction of patients and does not alter the autoimmune response. The more broadly used nonspecific immunosuppressive drugs work by inhibiting lymphocyte activation and proliferation but have little effect on long-lived plasma cells that are terminally differentiated and continue producing pathogenic antibodies [5, 6]. This may explain why treatment with nonspecific immunosuppressive drugs takes long time to show clinical improvement.

There are two major limitations in the current MG treatment. First, up to 10% of MG patients do not tolerate or are resistant to the available treatments [7]. Second, all immunosuppressant drugs, which are often used in the long-term control of chronic MG, inevitably carry the serious risks of infection and cancer. As such continued efforts have been put into searching for better MG treatment, as evident by the long list of clinical trials (ClinicalTrials.gov) testing well known immunosuppressive drugs such as methotrexate and azathioprine, as well as new biologics agents such as the anti-CD20 monoclonal antibody rituximab (which depletes B cells) and the anticomplement C5 monoclonal antibody eculizumab.

An ideal therapeutic approach to MG would be to inhibit the pathogenic autoimmune response to nAChR specifically without disrupting other functions of the immune system. Because nAChR is a dominant autoantigen in MG, it has served as the primary target for a wide range of studies attempting to develop antigen-specific therapy to induce immune tolerance to nAChR [8–14]. While some of these approaches showed promising results in animal model of experimental autoimmune MG (EAMG), translation to human MG treatment is uncertain. Furthermore, introducing an autoantigen like nAChR or its derivative peptides risks to inadvertently enhance the pathogenic autoimmune response.

Here, we will first review structural and molecular features of nAChR and its complexes with autoantibodies. Based on insights derived from structural studies, we will discuss several strategies to specifically inhibit the binding of pathological autoantibodies to nAChR or specifically eliminate nAChR-specific B cells.

2. Structural study of nAChR

As the first isolated neurotransmitter receptor and ion channel, nicotinic acetylcholine receptors (nAChRs) have been the focus of extensive studies to understand the basic mechanisms of neuronal signaling. These receptors are also being targeted for drug development against a variety of diseases, including addiction, depression, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, Alzheimer's disease, pain and inflammation [15]. nAChRs have been analyzed by a variety of biochemical, biophysical and electrophysiological experiments [16]. Tremendous efforts have been put into pursuing the atomic structure of nAChR. Electron microscopic analyses of nAChR from *Torpedo marmorata* by Unwin and colleagues have led to a 4 Å resolution model of the intact channel [17, 18], providing one of the most comprehensive structural model for nAChR. The structural details, however, are limited by the relatively low resolution. In this regard, the high-resolution structure of the acetylcholine binding protein (AChBP) published by Sixma and colleagues in 2001 was a major breakthrough [19]. AChBP shares ~24% sequence identity with nAChRs and has the same pentameric assembly. Its structures in different bound states have provided detailed information on the binding of a variety of agonists and

antagonists [20]. But AChBP does not function as an ion channel and may lack necessary structural features required for transmitting the ligand-binding signal across the protein body [21, 22]. The crystal structures of several prokaryotic homologues of nAChR have also been determined from different species and in different states [23–25]. These structures together with detailed biochemical and biophysical characterization have provided a great deal of insight into the fundamental mechanisms of ligand-dependent channel gating (reviewed in Corringer et al [26]). More recently, the structure of the anionic glutamate receptor (GluCl) from *C. elegans* [27], and human $\alpha 4\beta 2$ neuronal nicotinic receptor have also been determined [28]. However, direct structural information of mammalian muscle nAChRs at high resolution will be needed for further dissecting the mechanisms of neuromuscular junction signal transmission and for drug development against MG [29].

3. High-resolution structural analysis enabled by stabilizing nAChR mutants

Although large quantities of nAChR were available from *Torpedo* electric ray organ, crystallization was not successful, probably due to the heterogeneity of the protein samples prepared from the natural source. Heterologous expression in bacterial results in insoluble protein is due to the lack of proper post translation modifications such as glycosylation. Yeast *Pichia pastoris* has been a favorable expression system for overexpressing nAChR because of its mammalian-like glycosylation system. However, the expressed nAChR protein or extracellular domain (ECD) is often unstable, leading to aggregation and low yield [30, 31]. We have employed a number of strategies to overcome this difficulty, including expressing different family members of nAChR or its sub-domain (mostly ECD), constructing AChBP-nAChR chimera, and introducing specific mutations to enhance expression and stability [32]. Using the nAChR $\alpha 1$ as an example, we screened a PCR-generated mutant library of mouse nAChR $\alpha 1$ ECD for variants with increased expression and stability which led to the isolation of a triple mutant (V8E/W149R/V155A) that has much improved expression and stability than the wild type protein, and ultimately the determination of the crystal structure of nAChR $\alpha 1$ ECD bound to α -bungarotoxin at 1.94 Å resolution [22]. Structure comparison with the 4 Å electron microscopic model of nAChR and AChBP reveals that the isolated ECD is very similar to its counterpart in the intact channel and that the stabilizing mutations do not appear to alter the overall structure of the ECD.

All of the three mutations map to the surface of the protein (**Figure 1a**), with one (V8E) located on the N-terminal helix and the other two (W149R and V155A) located on loop B. The V8E mutation introduces a salt bridge with Lys84 (**Figure 1b**), whereas the W149R mutation introduces a salt bridge with Asp89 (**Figure 1c**). These salt bridges apparently contribute to protein stability as evident by the well-defined electron density of these exposed residues with long and charged side chains. Thus, the mutations seem to enhance the protein stability through at least two mechanisms. One is to remove surface exposed hydrophobic residues, including V155A (**Figure 1d**); the other is to introduce salt bridges on the protein surface. These observations suggest that the ECD of nAChR may be rationally engineered to improve solubility and stability. In principle, one can use homology models to guide the selection of exposed hydrophobic residues and to engineer surface salt bridges, which can increase the stability of recombinant mammalian nAChRs. This insight will be important for the design of stable chimeric nAChR antigen for specific targeting and elimination of nAChR-specific B cells (discussed further below).

Figure 1.

Mutations that stabilize nAChR $\alpha 1$ ECD. (a) The three mutations (boxed and indicated by arrow) are mapped on the surface of nAChR $\alpha 1$ ECD (dark green) and away from the binding site of α -bungarotoxin (orange) and the glycan (magenta); (b) the mutation Val8Glu establishes a salt bridge with Lys84. The surrounding structure is well ordered, showing well-defined electron density; (c) the mutation Trp149Arg establishes a salt bridge with Asp89. The side chains of both residues show well-defined electron density; (d) the mutation of Val155Ala removes an exposed hydrophobic residue. The surrounding structure is well ordered (Adapted from Chen [33]).

4. Functionally intrinsic instability of nAChR ECD

Most proteins have a densely packed hydrophobic core that is important for stable folding in aqueous solution. However, a hydration pocket was found inside the beta sandwich core of the nAChR $\alpha 1$ ECD [22]. This hydration pocket consists of two buried hydrophilic residues, Thr52 and Ser126, two ordered water molecules, and a few cavities, creating a packing defect near the disulfide that connects the two beta sheets. Both Thr52 and Ser126 are highly conserved in nAChRs but are substituted by large hydrophobic residues (Phe, Leu or Val) in the non-channel homologue AChBPs. This observation suggests that the nAChR ECD has evolved with a non-optimally packed core, hence predisposed to undergo conformational change during ligand-induced gating. Replacing Thr52 and Ser126 with their hydrophobic counterparts in AChBP significantly impaired the gating function of nAChR without affecting the folding of the protein structure [22]. This role of the hydration pocket on the conformation flexibility/dynamics of the nAChR ECD is supported by recent molecular dynamics studies [34]. This model also suggests that the specific location of the hydration cavity is important for a particular class of pentameric LGICs [35]. A practical implication of these observations is that one can design stabilization mutants of LGICs, including nAChR ECD, by structure-guided modifications of such packing defects, which are evolved for intrinsic ion channel functions but may be detrimental to recombinant production of proteins as therapeutic antigen.

5. Structural studies of the complexes between nAChR ECD and EAMG antibodies

Antibodies generated by the immune system may bind various epitopes on nAChR. It is therefore important to know if MG autoantibodies are randomly distributed to various epitopes and if they contribute equally or differently to the disease phenotype. This question is also therapeutically relevant if one wishes to use small molecules or single valent antibody [36] to block the binding of most

pathologically relevant autoantibodies to nAChR. Mammalian muscle nAChR has a pentameric structure composed of two $\alpha 1$, one $\beta 1$, one δ , and one ϵ (adult form) or γ (fetal form) subunit(s) [18]. Extensive studies suggest that autoantibodies to $\alpha 1$ play a major role in MG pathology [37–40]. Furthermore, more than half of all autoantibodies in MG and EAMG bind an overlapping region on the nAChR $\alpha 1$ subunit, known as the main immunogenic region (MIR) [41]. The MIR is defined by the ability of a single rat monoclonal antibody (mAb), mAb35, to inhibit the binding of about 65% autoantibodies from MG patients or rats with EAMG [42–44]. Subsequent studies have mapped MIR to a peptide region that spans residues 67–76 on nAChR $\alpha 1$ [45, 46]. Monoclonal antibodies directed to the MIR can passively transfer EAMG and possess all the key pathological functions of serum autoantibodies from MG patients [37]. Moreover, a recent study showed that titer levels of MIR-competing autoantibodies from MG patients, rather than the total amount of nAChR autoantibodies, correlate with disease severity [47]. These observations suggest that autoantibodies directed to the MIR on nAChR $\alpha 1$ play a major role in the pathogenesis of MG [41]. However, autoantibodies classified as MIR-directed by competition assay may not necessarily have the same binding mechanisms to nAChR: two MIR-competing autoantibodies may share common or overlapping epitopes or may bind different epitopes but compete through steric effect [14].

Given their established myasthenogenic role, extensive efforts have been put into characterizing the interactions between MG autoantibodies and nAChR using biochemical [45, 46, 48–53], structural [22, 54–56], and modeling approaches [57]. More recently, the first crystal structures of human (pdb code: 5HBT) and mouse (pdb code: 5HBV) nAChR ECD bound by the Fab fragment of an EAMG autoantibody, Fab35 were determined [58]. Both crystal structures are very similar, so the discussion here will focus mainly on the human complex (pdb code: 5HBT). The crystal structure, which also contains α -Btx that binds and stabilizes nAChR ECD to facilitate crystallization, shows that Fab35 binds to nAChR $\alpha 1$ in an upright orientation, away from the α -Btx (**Figure 2**). The Fab35 binding sites on nAChR $\alpha 1$ include the MIR and the N-terminal helix. Fab35 has the canonical IgG antibody structure where the complementarity determining regions (CDRs) from the heavy chain, CDR-H2 and CDR-H3, and the light chain, CDR-L3, form the binding site for nAChR $\alpha 1$. Contacting residues from Fab35 and nAChR $\alpha 1$ (defined as being closer than 4.5 Å) can be mapped using the crystal structure. Such contacting analysis revealed several “hotspots” on nAChR $\alpha 1$ that make numerous contacts to Fab35, including Asn68 and Asp71 from the MIR loop and Arg6 and Lys10 from the N-terminal helix. As shown in **Figure 3**, each of these four “hotspots” anchors an extensive network of interactions that display remarkable chemical complementarities. The importance of these hotspots are supported by extensive mutagenesis studies [50, 51, 53, 59], which showed that Asn68 and Asp71 of the MIR are essential for MG autoantibody binding, while the surrounding Pro69 and Tyr72, when mutated, also affect the interaction between the antibody and the receptor. Mutation of N68D and D71K in the intact receptor also suggested Asn68 and Asp71 are of vital importance for the interaction [49]. On the N-terminal helix of *Torpedo* nAChR $\alpha 1$, two exposed residues, Arg6 and Asn10, which correspond to Arg6 and Lys10 in human nAChR $\alpha 1$, respectively, are found to be critical to MG antibody binding by mutational analyses [53]. Many nAChR residues found to be important for antibody binding by mutagenesis studies, including Asn68 and Asp71 of the MIR and Arg6 and Lys10 of the N-terminal helix, were indeed found to be interaction “hotspots” at the Fab35/nAChR $\alpha 1$ interface. More recent studies using natively folded nAChR $\alpha 1\alpha 7$ chimera proteins [52] or GFP-fused protein fragments [53] showed that the N-terminal helix (residues 1–14) and the nearby loop region (residues 15–32) are also important for high affinity MG antibody binding. These biochemical observations are in excellent agreement with the binding interface structure observed in the crystals (**Figure 2**).

Figure 2. Crystal structure of the ternary complex of nAChR $\alpha 1$ ECD bound by Fab35 and α -Btx. (a) Ribbon representation of nAChR $\alpha 1$ ECD ($\alpha 1$: cyan) in complex with α -Btx (green) and Fab35 (heavy chain (H, yellow) and light chain (L, magenta)). The variable domains (V_H and V_L) and the constant domains (C_H and C_L) of the antibody are indicated accordingly. (b) Surface representation of the ternary complex. (c) Zoomed-in view of the binding interface. The complementarity determining regions of the heavy chain and light chain are indicated as H1, H2, H3, L1, L2, and L3, respectively (Adapted from Noridomi et al. [58]).

Figure 3. Detailed interactions between Fab35 and nAChR $\alpha 1$ ECD at the binding interface. (a) Binding interactions at the vicinity of Asp71 of $\alpha 1$ (located at the MIR). (b) Interactions at the vicinity of Asn68 of $\alpha 1$ (located at the MIR). (c) Interactions involving Arg6 and Lys10 of $\alpha 1$ (located at the N-terminus of $\alpha 1$). (d) Interactions mediated by His3 of $\alpha 1$ (located at the N-terminus of $\alpha 1$) (Adapted from Noridomi et al. [58]).

Although biochemical mapping of antibody-binding residues on nAChR $\alpha 1$ were performed with different antibodies (e.g., mAb210 and mAb132A) [45, 46, 48–53], it is remarkable that these biochemical data agree so well with the crystal structure. The fact that many MIR residues at the center of the antibody-receptor interface are important for the high affinity binding of a variety of MG antibodies suggests that many MIR-directed autoantibodies share similar binding mechanisms to the

core MIR/N-helix region. This is a rather surprising finding given the potential heterogeneity of nAChR antibodies mentioned above. An important implication of this finding is that it may be possible to find small molecule inhibitors to block the binding of a large fraction of pathological MG autoantibodies to nAChR.

6. Structural comparison of Fab35 with other MG autoantibodies

To see how various MG/EAMG mAbs may bind nAChR through similar or different mechanisms, we compared the structure of Fab35 with that of two other MG mAbs (Fab198: pdb code 1FN4 and Fab192: pdb code, 1C5D) that have been determined previously [55, 56]. Superposition of the structure of Fab198 and Fab35 from the ternary complex shows that these two Fabs share a similar antigen-binding site (**Figure 4a**). As such, the MIR loop fits snugly into the pocket formed by the CDR-H2, CDR-H3 and CDR-L3 loops of Fab198, as predicted by previous modeling studies [57]. The CDR-H2 loop of Fab198 is also in a position to interact with the N-terminal α -helix adjacent to the MIR (**Figure 4b**). Even more remarkably, many key α 1-binding residues in Fab35 are also conserved in Fab198 and they appear to have similar contacts to nAChR α 1 in the modeled Fab198/nAChR α 1 binding interface (**Figure 4b**). These residues include Trp47 from CDR-H2, Arg50 from CDR-H2, and Tyr95 from CDR-L3 at the center of the MIR-binding pocket, and Trp52 and Asp54 (both from CDR-H2) which interact with the N-terminal α -helix. In contrast to the structural similarities shown above, the CDR-H3 loops between Fab198 and Fab35 differ significantly in length and sequence. The CDR-H3 loop of Fab198 is too short to interact with the surface pocket of nAChR α 1, which is occupied by the corresponding CDR-H3 loop of Fab35 in the complex crystal structure (**Figure 4b**). These structural analyses suggest that mAb35 and mAb198 share a high degree of similarity in binding mechanism to the core MIR/N-terminal helix region but differ in the periphery of the binding interface. On the other hand, superposition of the structure of Fab192 onto that of Fab35 in the ternary complex reveals substantial differences (not shown here). The variable domains (V_H and V_L) have a significant rotational twist, such that the MIR loop does not fit into the antigen-binding site of Fab192. What is more, the key α 1-binding residues of Fab35, like Arg50 and Trp52 of CDR-H2, are not conserved in Fab192. These structural differences suggest that Fab192 may differ significantly from Fab35 in terms of binding mechanisms to nAChR α 1, confirming and extending the differences previously recognized between the two [52].

Figure 4.
 Structural comparisons among MG mAbs. (a) Superposition of Fab198 [55] (heavy chain: purple and light green; light chain: dark green) onto Fab35 in the Fab35/nAChR α 1/ α -Btx ternary complex using the Ca backbone.
 (b) Detailed comparison of the binding interface. The residues are colored according to their protein subunits.

7. MG autoantibody repertoire and MIR-directed autoantibodies

A number of studies showed that the total amount of nAChR antibodies in the serum of MG patients does not seem to correlate with disease severity, suggesting that various nAChR antibodies that bind different regions on nAChR may contribute differently to this disease [41, 60–62]. As discussed above, the total amount of autoantibody from MG patients directed to the MIR of nAChR $\alpha 1$ subunit did show significant correlation with disease severity [47]. These observations suggest that autoantibodies directed to nAChR $\alpha 1$ MIR play a major role in the pathogenesis of MG [41]. It is now clear that many MIR-directed autoantibodies bind a composite epitope consisting of the original MIR ($\alpha 1$, 67–76) and the N-terminal helix ($\alpha 1$, 2–14) (N-helix) and surrounding regions ($\alpha 1$, 15–32). The structural analyses above and published biochemical data suggest that some MIR-directed autoantibodies (e.g., mAb35 and mAb198) bind epitopes centered around the MIR/N-helix core region while others (e.g., mAb192) seems to require epitopes outside the MIR/N-helix core. Nevertheless, based on crystallography studies and structure-guided analyses of existing biochemical data, it can be concluded that despite the heterogeneity of MG autoantibody repertoire a large fraction of MG autoantibodies share a highly-conserved binding mechanism to a core region on the nAChR, suggesting that it is possible to use a single or a limited set of small molecules to block the binding of a large fraction of MG autoantibodies. Because MG autoantibodies directed to the MIR region on nAChR are most relevant to the MG disease, MIR and its surrounding region are therefore an attractive target site for developing small molecules to block the binding of MG autoantibodies. Blocking the binding of MG autoantibodies to nAChR will likely have a direct impact on the antibody-mediated pathologies and may even alter the long-term immune response to nAChR in MG patient.

8. Small molecules blocking the binding of MIR-directed autoantibody to nAChR

Targeting protein-protein interface for drug development is generally more challenging than the enzyme active sites [63]. This is especially true for flat protein interfaces lacking features for small molecule binding. However, successes have been achieved with a number of well-known targets, including the p53/MDM2 complex [64], the Bcl-xL/Bak complex [65] and the IL2/IL2R complex [66, 67]. A common feature of these complexes is that the protein-protein binding interfaces contain concave pockets lined with hydrophobic residues, which may provide favorable anchoring points for small molecules to bind and compete with protein-protein interactions. The crystal structure of the Fab35/nAChR $\alpha 1$ complex revealed that their binding interface is characterized by mutual insertions of loops into the pockets of binding partners. On the receptor side (**Figure 5**), the MIR loop inserts deeply into a surface pocket between V_H and V_L , and the N-terminal α -helix sits into a groove on the surface of V_H . On the antibody side (**Figure 6**), the CDR-H3 protrudes into a surface pocket formed by the N-terminal α -helix, the loop following the N-terminal α -helix, the MIR and the loop preceding the MIR (referred to as the CDRH3 pocket here after). Based on these structural features, two MG inhibitor design strategies can be envisioned. One is to find small molecules that bind the surface pockets on Fab35 (**Figure 5**). But this approach faces the potential issue of antibody heterogeneity in sera of human MG patients because small molecule inhibitors may bind some but not other pathological autoantibodies, as it is highly possible antibodies binding to the same epitope may have subtle differences in their antigen-binding site structures. Another approach is to find small molecules to bind the CDRH3 pocket on nAChR (**Figure 6**). Small molecules bound to this site will directly interfere with the binding

Figure 5.
Surface pockets on Fab35 bound by the nAChR MIR loop (white dashed circle) and the N-terminal helix (black dashed circle).

Figure 6.
The surface pocket (green dashed circle) on nAChR $\alpha 1$ bound by the CDR-H3 loop from Fab35 (indicated as H3 in the figure).

of mAb35 by competing with its CDR-H3. Even for other mAbs with short CDR-H3, such as mAb198, the compounds may also block the binding of CDR-H3 through steric hindrances. Moreover, since the CDRH3 pocket is immediate adjacent (about 6–8 Å) (**Figure 6**) to the MIR/N-helix core region critical for the binding of a large group of MG autoantibodies, compounds bound to CDRH3 could sterically and/or allosterically inhibit the binding of most pathological MG autoantibodies efficiently. Because of its concaved structure, CDRH3 pocket could serve as the anchoring point to design and/or screen small molecules that bind nAChR $\alpha 1$ and complete with MG autoantibodies directed to MIR and its nearby regions.

9. nAChR-specific B cell inhibition and depletion with engineered antigen chimera

The fact that pathogenic B cell clones can populate for a long time in patients' body may explain why MG is usually a chronic disease. Ectopic germinal centers are found

in the thymus of many MG patients who are diagnosed with thymoma or thymus hyperplasia, where nAChR-specific B lymphocyte are constantly activated, selected and matured to produce the antibody, leading to the disease [68]. This disease model underlies the rationale of thymectomy as a widely adopted treatment of MG, but the result varies depending on the subtype of the disease, with a complete remission rate of 25–53% [69]. These results suggest there are possibly other unknown sites where nAChR specific B cells are activated, selected and matured [13].

Using B cell surface marker CD20 [70–72] or possibly CD19 [73] as the target, disease-causing B cells can be depleted at the cost of killing normal B cells. For example, an ongoing clinical trial, NCT02110706, is testing if rituximab, which targets CD20 on B cells, can be a safe and beneficial therapeutics for MG. In general, treatment with B cell depletion agent often requires a long recovery time before B cells return to normal level again [71]. Moreover, the treatment has been reported to have a short effective duration time for MuSK-positive MG [74]. Long-term usage of such agent may compromise immunological function with increased risk of infection such as Progressive multifocal leukoencephalopathy (PML) and malignancy [72]. As such, strategies targeting nAChR specific B cells seem to be attractive. Since each B cell expresses B cell Receptors of the same idiotype as its secreted antibody on its surface, one can use such property to specifically target autoreactive B cell as long as the antigenicity of the autoimmune disease is clear. The idea was borrowed from immunotoxins [75] in which an antigen-toxin chimera was constructed. The antigen moiety is used to target the B cells that express the BCR of the same idiotype as the antibody and the toxin moiety is responsible for conveying death signal to the target B cells. In a pioneering study in 1983 the author fused thymoglobulin with ricin to treat an autoimmune disorder-Hashimoto's thyroiditis [76]. Another attempt was tried a decade later in another autoimmune disease-Pemphigus Vulgaris, in which the authors constructed antigen-toxin fusion protein that can specifically target Dsg3-specific hybridoma cells [77]. Similar strategies have also been attempted in the treatment of MG. In a study of 2006, the author fused the nAChR $\alpha 1$ ECD to a plant toxin and showed its effectiveness in specifically killing of $\alpha 1$ -specific B cells [78]. More recently, researchers have developed a variant of such strategy in which nAChR $\alpha 1$ ECD was fused with Fc domain of antibody, which was used to convey the negative signal, since B cells express and only express one kind of Fc receptor, namely Fc γ RIIB, which transduce negative signal for B cell activation. Consequently, such chimeric protein will specifically target the nAChR $\alpha 1$ specific B cell via the binding to the BCR and deliver negative signal to inhibit $\alpha 1$ specific B cells [79, 80].

The idea of antigen-chimera in the treatment of MG seems attractive but will not be practical unless the chimeric protein is stable enough to be used as a therapeutic agent. As mentioned above, nAChR $\alpha 1$ is just one subunit of the nAChR pentamer and is intrinsically unstable, making the expression of wild type nAChR $\alpha 1$ ECD in stable soluble form very challenging. However, as discussed earlier in this chapter, crystallography studies of nAChR $\alpha 1$ ECD in recent years have accumulated extensive experience and knowledge in designing strategic mutations to improve the stability and expression level of nAChR $\alpha 1$ ECD protein while preserve the binding of MIR-directed MG autoantibodies [22, 31, 58] These progresses will greatly facilitate the approach to using engineered antigen chimera to specially inhibit and eliminate nAChR-specific B cells for MG treatment.

10. Outlook

Insights from structural studies and molecular biology/biochemical analyses may ultimately lead to precision medicine and personalized treatment of MG by

antigen profiling of patient and the use of corresponding molecular missiles to eliminate antigen specific antibodies or B-cells, induce antigen specific tolerance, or blocking nAChR-autoantibody binding by small molecules. These approaches, once established in the treatment of MG, could be expanded to other autoimmune diseases with well-defined antigen targets.

IntechOpen

IntechOpen

Author details

Jiang Xu, Kaori Noridomi and Lin Chen*
Molecular and Computational Biology, Departments of Biological Sciences and
Chemistry, University of Southern California, Los Angeles, CA, USA

*Address all correspondence to: linchen@usc.edu

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Philips L. The epidemiology of myasthenia gravis. *Annals of the New York Academy of Sciences*. 2003;**998**:407-412. DOI: 10.1196/annals.1254.053
- [2] Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nature Medicine*. 2001;**7**:365. DOI: 10.1038/85520
- [3] Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Archives of Neurology*. 2012;**69**:445-451. DOI: 10.1001/archneurol.2011.2393
- [4] Vincent A, Palace J, Hilton-Jones D. Myasthenia gravis. *Lancet (London, England)*. 2001;**357**:2122-2128. DOI: 10.1016/S0140-6736(00)05186-2
- [5] Gomez AM, Vrolix K, Martínez-Martínez P, Molenaar PC, Phernambucq M, van der Esch E, et al. Proteasome inhibition with bortezomib depletes plasma cells and autoantibodies in experimental autoimmune myasthenia gravis. *Journal of Immunology*. 2011;**186**:2503-2513. DOI: 10.4049/JIMMUNOL.1002539
- [6] Gomez AM, Willcox N, Molenaar PC, Buurman W, Martinez-Martinez P, De Baets MH, et al. Targeting plasma cells with proteasome inhibitors: Possible roles in treating myasthenia gravis? *Annals of the New York Academy of Sciences*. 2012;**1274**:48-59. DOI: 10.1111/j.1749-6632.2012.06824.x
- [7] Gold R, Dalakas MC, Toyka KV. Immunotherapy in autoimmune neuromuscular disorders. *Lancet Neurology*. 2003;**2**:22-32. DOI: 10.1016/S1474-4422(03)00264-3
- [8] Nicolle MW, Nag B, Sharma SD, Willcox N, Vincent A, Ferguson DJ, et al. Specific tolerance to an acetylcholine receptor epitope induced in vitro in myasthenia gravis CD4+ lymphocytes by soluble major histocompatibility complex class II-peptide complexes. *The Journal of Clinical Investigation*. 1994;**93**:1361-1369. DOI: 10.1172/JCI117112
- [9] Okumura S, McIntosh K, Drachman DB. Oral administration of acetylcholine receptor: Effects on experimental myasthenia gravis. *Annals of Neurology*. 1994;**36**:704-713. DOI: 10.1002/ana.410360504
- [10] Karachunski PI, Ostlie NS, Okita DK, Garman R, Conti-Fine BM. Subcutaneous administration of T-epitope sequences of the acetylcholine receptor prevents experimental myasthenia gravis. *Journal of Neuroimmunology*. 1999;**93**:108-121. DOI: 10.1016/S0165-5728(98)00208-2
- [11] Pass-Rozner M, Faber-Elmann A, Sela M, Mozes E. Immunomodulation of myasthenia gravis associated autoimmune responses by an altered peptide ligand: Mechanisms of action. In: *Myasthenia gravis*. Dordrecht: Springer Netherlands; 2000. pp. 182-194. DOI: 10.1007/978-94-011-4060-7_17
- [12] Wu B, Golusko E, Christadoss P. Experimental autoimmune myasthenia gravis in the mouse. In: *Current Protocols in Immunology*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2001. pp. 15.8.1-15.8.19. DOI: 10.1002/0471142735.im1508s21
- [13] Yi H-J, Chae C-S, So J-S, Tzartos SJ, Souroujon MC, Fuchs S, et al. Suppression of experimental myasthenia gravis by a B-cell epitope-free recombinant acetylcholine receptor. *Molecular Immunology*.

- 2008;**46**:192-201. DOI: 10.1016/J.MOLIMM.2008.08.264
- [14] Luo J, Lindstrom J. AChR-specific immunosuppressive therapy of myasthenia gravis. *Biochemical Pharmacology*. 2015;**97**:609-619. DOI: 10.1016/J.BCP.2015.07.011
- [15] Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: From basic science to therapeutics. *Pharmacology & Therapeutics*. 2013;**137**:22-54. DOI: 10.1016/J.PHARMTHERA.2012.08.012
- [16] Sine SM, Engel AG. Recent advances in Cys-loop receptor structure and function. *Nature*. 2006;**440**:448-455. DOI: 10.1038/nature04708
- [17] Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature*. 2003;**423**:949-955. DOI: 10.1038/nature01748
- [18] Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *Journal of Molecular Biology*. 2005;**346**:967-989. DOI: 10.1016/j.jmb.2004.12.031
- [19] Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, Smit AB, et al. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature*. 2001;**411**:269-276. DOI: 10.1038/35077011
- [20] Rucktooa P, Smit AB, Sixma TK. Insight in nAChR subtype selectivity from AChBP crystal structures. *Biochemical Pharmacology*. 2009;**78**:777-787. DOI: 10.1016/J.BCP.2009.06.098
- [21] Karlin A. A touching picture of nicotinic binding. *Neuron*. 2004;**41**:841-842. DOI: 10.1016/S0896-6273(04)00151-5
- [22] Dellisanti CD, Yao Y, Stroud JC, Wang Z-Z, Chen L. Crystal structure of the extracellular domain of nAChR $\alpha 1$ bound to α -bungarotoxin at 1.94 Å resolution. *Nature Neuroscience*. 2007;**10**:953-962. DOI: 10.1038/nn1942
- [23] Hilf RJC, Dutzler R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature*. 2008;**452**:375-379. DOI: 10.1038/nature06717
- [24] Hilf RJC, Dutzler R. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. *Nature*. 2009;**457**:115-118. DOI: 10.1038/nature07461
- [25] Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux J-P, Delarue M, et al. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature*. 2009;**457**:111-114. DOI: 10.1038/nature07462
- [26] Corringer P-J, Poitevin F, Prevost MS, Sauguet L, Delarue M, Changeux J-P. Structure and pharmacology of pentameric receptor channels: From bacteria to brain. *Structure*. 2012;**20**:941-956. DOI: 10.1016/J.STR.2012.05.003
- [27] Hibbs RE, Gouaux E. Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature*. 2011;**474**:54-60. DOI: 10.1038/nature10139
- [28] Morales-Perez CL, Noviello CM, Hibbs RE. X-ray structure of the human $\alpha 4\beta 2$ nicotinic receptor. *Nature*. 2016;**538**:411-415. DOI: 10.1038/nature19785
- [29] Taly A, Corringer P-J, Guedin D, Lestage P, Changeux J-P. Nicotinic receptors: Allosteric transitions and therapeutic targets in the nervous system. *Nature Reviews. Drug Discovery*. 2009;**8**:733-750. DOI: 10.1038/nrd2927
- [30] Psaridi-Linardaki L, Mamalaki A, Remoundos M, Tzartos SJ. Expression

of soluble ligand- and antibody-binding extracellular domain of human muscle acetylcholine receptor alpha subunit in yeast *Pichia pastoris*. Role of glycosylation in alpha-bungarotoxin binding. *The Journal of Biological Chemistry*. 2002;277:26980-26986. DOI: 10.1074/jbc.M110731200

[31] Yao Y, Wang J, Viroonchatapan N, Samson A, Chill J, Rothe E, et al. Yeast expression and NMR analysis of the extracellular domain of muscle nicotinic acetylcholine receptor alpha subunit. *The Journal of Biological Chemistry*. 2002;277:12613-12621. DOI: 10.1074/jbc.M108845200

[32] Zouridakis M, Zisimopoulou P, Poulas K, Tzartos SJ. Recent advances in understanding the structure of nicotinic acetylcholine receptors. *IUBMB Life*. 2009;61:407-423. DOI: 10.1002/iub.170

[33] Chen L. In pursuit of the high-resolution structure of nicotinic acetylcholine receptors. *The Journal of Physiology*. 2010;588:557-564. DOI: 10.1113/jphysiol.2009.184085

[34] Cheng X, Ivanov I, Wang H, Sine SM, McCammon JA. Molecular-dynamics simulations of ELIC—A prokaryotic homologue of the nicotinic acetylcholine receptor. *Biophysical Journal*. 2009;96:4502-4513. DOI: 10.1016/j.bpj.2009.03.018

[35] Dellisanti CD, Hanson SM, Chen L, Czajkowski C. Packing of the extracellular domain hydrophobic core has evolved to facilitate pentameric ligand-gated ion channel function. *The Journal of Biological Chemistry*. 2011;286:3658-3670. DOI: 10.1074/jbc.M110.156851

[36] Tsantili P, Tzartos SJ, Mamalaki A. High affinity single-chain Fv antibody fragments protecting the human nicotinic acetylcholine receptor. *Journal of Neuroimmunology*. 1999;94:15-27. DOI: 10.1016/S0165-5728(98)00195-7

[37] Tzartos S, Hochschwender S, Vasquez P, Lindstrom J. Passive transfer of experimental autoimmune myasthenia gravis by monoclonal antibodies to the main immunogenic region of the acetylcholine receptor. *Journal of Neuroimmunology*. 1987;15:185-194. DOI: 10.1016/0165-5728(87)90092-0

[38] Sideris S, Lagoumintzis G, Kordas G, Kostelidou K, Sotiriadis A, Poulas K, et al. Isolation and functional characterization of anti-acetylcholine receptor subunit-specific autoantibodies from myasthenic patients: Receptor loss in cell culture. *Journal of Neuroimmunology*. 2007;189:111-117. DOI: 10.1016/J.JNEUROIM.2007.06.014

[39] Tzartos SJ, Bitzopoulou K, Gavra I, Kordas G, Jacobson L, Kostelidou K, et al. Antigen-specific apheresis of pathogenic autoantibodies from myasthenia gravis sera. *Annals of the New York Academy of Sciences*. 2008;1132:291-299. DOI: 10.1196/annals.1405.017

[40] Kordas G, Lagoumintzis G, Sideris S, Poulas K, Tzartos SJ. Direct proof of the in vivo pathogenic role of the AChR autoantibodies from myasthenia gravis patients. *PLoS One*. 2014;9:e108327. DOI: 10.1371/journal.pone.0108327

[41] Tzartos SJ, Barkas T, Cung MT, Mamalaki A, Marraud M, Orlewski P, et al. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunological Reviews*. 1998;163:89-120. DOI: 10.1111/j.1600-065X.1998.tb01190.x

[42] Tzartos SJ, Lindstrom JM. Monoclonal antibodies used to probe acetylcholine receptor structure: Localization of the main immunogenic region and detection of similarities between subunits. *Proceedings of the National Academy of Sciences*.

1980;77:755-759. DOI: 10.1073/PNAS.77.2.755

[43] Tzartos SJ, Seybold ME, Lindstrom JM. Specificities of antibodies to acetylcholine receptors in sera from myasthenia gravis patients measured by monoclonal antibodies. Proceedings of the National Academy of Sciences of the United States of America. 1982;79:188. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC345688/> [Accessed: December 27, 2018]

[44] Tzartos S, Langeberg L, Hochschwender S, Lindstrom J. Demonstration of a main immunogenic region on acetylcholine receptors from human muscle using monoclonal antibodies to human receptor. FEBS Letters. 1983;158:116-118. DOI: 10.1016/0014-5793(83)80688-7

[45] Barkas T, Gabriel JM, Mauron A, Hughes GJ, Roth B, Alliod C, et al. Monoclonal antibodies to the main immunogenic region of the nicotinic acetylcholine receptor bind to residues 61-76 of the alpha subunit. The Journal of Biological Chemistry. 1988;263:5916-5920. <http://www.ncbi.nlm.nih.gov/pubmed/2451673> [Accessed: December 27, 2018]

[46] Tzartos SJ, Kokla A, Walgrave SL, Conti-Tronconi BM. Localization of the main immunogenic region of human muscle acetylcholine receptor to residues 67-76 of the alpha subunit. Proceedings of the National Academy of Sciences of the United States of America. 1988;85:2899-2903. <http://www.ncbi.nlm.nih.gov/pubmed/3362855> [Accessed: December 27, 2018]

[47] Masuda T, Motomura M, Utsugisawa K, Nagane Y, Nakata R, Tokuda M, et al. Antibodies against the main immunogenic region of the acetylcholine receptor correlate with disease severity in myasthenia gravis.

Journal of Neurology, Neurosurgery, and Psychiatry. 2012;83:935-940. DOI: 10.1136/jnnp-2012-302705

[48] Das MK, Lindstrom J. The main immunogenic region of the nicotinic acetylcholine receptor: Interaction of monoclonal antibodies with synthetic peptides. Biochemical and Biophysical Research Communications. 1989;165:865-871. DOI: 10.1016/S0006-291X(89)80046-4

[49] Saedi MS, Anand R, Conroy WG, Lindstrom J. Determination of amino acids critical to the main immunogenic region of intact acetylcholine receptors by in vitro mutagenesis. FEBS Letters. 1990;267:55-59. DOI: 10.1016/0014-5793(90)80286-R

[50] Papadouli I, Potamianos S, Hadjidakis I, Bairaktari E, Tsikaris V, Sakarellos C, et al. Antigenic role of single residues within the main immunogenic region of the nicotinic acetylcholine receptor. The Biochemical Journal. 1990;269:239-245. DOI: 10.1042/BJ2690239

[51] Papadouli I, Sakarellos C, Tzartos SJ. High-resolution epitope mapping and fine antigenic characterization of the main immunogenic region of the acetylcholine receptor: Improving the binding activity of synthetic analogues of the region. European Journal of Biochemistry. 1993;211:227-234. DOI: 10.1111/j.1432-1033.1993.tb19890.x

[52] Luo J, Taylor P, Losen M, De Baets MH, Shelton GD, Lindstrom J. Neurobiology of disease main immunogenic region structure promotes binding of conformation-dependent myasthenia gravis autoantibodies, nicotinic acetylcholine receptor conformation maturation, and agonist sensitivity. 2009;29(44):13898-13908. DOI: 10.1523/JNEUROSCI.2833-09.2009

[53] Morell SW, Trinh VB, Gudipati E, Friend A, Page NA, Agius MA, et al.

Structural characterization of the main immunogenic region of the Torpedo acetylcholine receptor. *Molecular Immunology*. 2014;**58**:116-131. DOI: 10.1016/J.MOLIMM.2013.11.005

[54] Beroukhim R, Unwin N. Three-dimensional location of the main immunogenic region of the acetylcholine receptor. *Neuron*. 1995;**15**:323-331. DOI: 10.1016/0896-6273(95)90037-3

[55] Kontou M, Leonidas DD, Vatzaki EH, Tsantili P, Mamalaki A, Oikonomakos NG, et al. The crystal structure of the Fab fragment of a rat monoclonal antibody against the main immunogenic region of the human muscle acetylcholine receptor. *European Journal of Biochemistry*. 2000;**267**:2389-2397. DOI: 10.1046/j.1432-1327.2000.01252.x

[56] Poulas K, Eliopoulos E, Vatzaki E, Navaza J, Kontou M, Oikonomakos N, et al. Crystal structure of Fab198, an efficient protector of the acetylcholine receptor against myasthenogenic antibodies. *European Journal of Biochemistry*. 2001;**268**:3685-3693. DOI: 10.1046/j.1432-1327.2001.02274.x

[57] Kleinjung J, Petit M-C, Orlewski P, Mamalaki A, Tzartos SJ, Tsikaris V, et al. The third-dimensional structure of the complex between an Fv antibody fragment and an analogue of the main immunogenic region of the acetylcholine receptor: A combined two-dimensional NMR, homology, and molecular modeling approach. *Biopolymers*. 2000;**53**: 113-128. DOI: 10.1002/(SICI)1097-0282(200002)53:2<113: AID-BIP1>3.0.CO;2-J

[58] Noridomi K, Watanabe G, Hansen MN, Han GW, Chen L. Structural insights into the molecular mechanisms of myasthenia gravis and their therapeutic implications. *eLife*. 2017;**6**. DOI: 10.7554/eLife.23043

[59] Bellone M, Tang F, Milius R, Conti-Tronconi BM. The main immunogenic region of the nicotinic acetylcholine receptor. Identification of amino acid residues interacting with different antibodies. *Journal of Immunology*. 1989;**143**:3568-3579. <http://www.ncbi.nlm.nih.gov/pubmed/2584708> [Accessed: December 27, 2018]

[60] Mossman S, Vincent A, Newsom-Davis J. Passive transfer of myasthenia gravis by immunoglobulins: Lack of correlation between AChR with antibody bound, acetylcholine receptor loss and transmission defect. *Journal of the Neurological Sciences*. 1988;**84**:15-28. DOI: 10.1016/0022-510X(88)90170-0

[61] Somnier FE. Clinical implementation of anti-acetylcholine receptor antibodies. *Journal of Neurology, Neurosurgery, and Psychiatry*. 1993;**56**:496-504. <http://www.ncbi.nlm.nih.gov/pubmed/8505642> [Accessed: December 27, 2018]

[62] Berrih-Aknin S. Myasthenia gravis, a model of organ-specific autoimmune disease. *Journal of Autoimmunity*. 1995;**8**:139-143. DOI: 10.1006/JAUT.1995.0011

[63] Scott DE, Bayly AR, Abell C, Skidmore J. Small molecules, big targets: Drug discovery faces the protein-protein interaction challenge. *Nature Reviews. Drug Discovery*. 2016;**15**: 533-550. DOI: 10.1038/nrd.2016.29

[64] Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. 2004;**303**:844-848. DOI: 10.1126/science.1092472

[65] Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature*

Medicine. 2013;**19**:202-208. DOI: 10.1038/nm.3048

[66] Rickert M, Wang X, Boulanger MJ, Goriatcheva N, Garcia KC. The structure of interleukin-2 complexed with its alpha receptor. *Science*. 2005;**308**:1477-1480. DOI: 10.1126/science.1109745

[67] Wilson CGM, Arkin MR. Small-Molecule Inhibitors of IL-2/IL-2R: Lessons Learned and Applied. Berlin, Heidelberg: Springer; 2010. pp. 25-59. DOI: 10.1007/82_2010_93

[68] Sims GP, Shiono H, Willcox N, Stott DI. Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. *Journal of Immunology*. 2001;**167**:1935-1944. DOI: 10.4049/JIMMUNOL.167.4.1935

[69] Venuta F, Rendina EA, De Giacomo T, Della Rocca G, Antonini G, Ciccone AM, et al. Thymectomy for myasthenia gravis: A 27-year experience. *European Journal of Cardio-Thoracic Surgery*. 1999;**15**:621-624. Discussion 624-625. <http://www.ncbi.nlm.nih.gov/pubmed/10386407> [Accessed: December 27, 2018]

[70] Dupuy A, Viguier M, Bédane C, Cordoliani F, Blaise S, Aucouturier F, et al. Treatment of refractory pemphigus vulgaris with rituximab (anti-CD20 monoclonal antibody). *Archives of Dermatology*. 2004;**140**: 91-96. DOI: 10.1001/archderm.140.1.91

[71] Yi JS, DeCroos EC, Sanders DB, Weinhold KJ, Gupitill JT. Prolonged B-cell depletion in MuSK myasthenia gravis following rituximab treatment. *Muscle & Nerve*. 2013;**48**:992-993. DOI: 10.1002/mus.24063

[72] Du FH, Mills EA, Mao-Draayer Y. Next-generation anti-CD20 monoclonal antibodies in autoimmune disease treatment. *Autoimmunity*

Highlights. 2017;**8**:12. DOI: 10.1007/s13317-017-0100-y

[73] Chen D, Gallagher S, Monson N, Herbst R, Wang Y. Inebilizumab, a B cell-depleting anti-CD19 antibody for the treatment of autoimmune neurological diseases: Insights from preclinical studies. *Journal of Clinical Medicine*. 2016;**5**:107. DOI: 10.3390/jcm5120107

[74] Stathopoulos P, Kumar A, Nowak RJ, O'Connor KC. Autoantibody-producing plasmablasts after B cell depletion identified in muscle-specific kinase myasthenia gravis. *JCI Insight*. 2017;**2**(17):e94263 DOI: 10.1172/jci.insight.94263

[75] Madhumathi J, Verma RS. Therapeutic targets and recent advances in protein immunotoxins. *Current Opinion in Microbiology*. 2012;**15**:300-309. DOI: 10.1016/J.MIB.2012.05.006

[76] Rennie DP, McGregor AM, Wright J, Weetman AP, Hall R, Thorpe P. An immunotoxin of ricin A chain conjugated to thyroglobulin selectively suppresses the antithyroglobulin autoantibody response. *Lancet (London, England)*. 1983;**2**:1338-1340. <http://www.ncbi.nlm.nih.gov/pubmed/6139673> [Accessed: December 27, 2018]

[77] Proby CM, Ota T, Suzuki H, Koyasu S, Gamou S, Shimizu N, et al. Development of chimeric molecules for recognition and targeting of antigen-specific B cells in pemphigus vulgaris. *The British Journal of Dermatology*. 2000;**142**:321-330. DOI: 10.1046/j.1365-2133.2000.03328.x

[78] Hossann M, Li Z, Shi Y, Kreilinger U, Büttner J, Vogel PD, et al. Novel immunotoxin: A fusion protein consisting of gelonin and an acetylcholine receptor fragment as a potential immunotherapeutic agent for the treatment of Myasthenia gravis. *Protein Expression and Purification*.

2006;**46**:73-84. DOI: 10.1016/J.
PEP.2005.08.029

[79] Chang T, Lin H, Gao J, Li W, Xu J, Sun CJ, et al. Selective recognition and elimination of nicotinic acetylcholine receptor-reactive B cells by a recombinant fusion protein AChR-Fc in myasthenia gravis in vitro. *Journal of Neuroimmunology*. 2010;**227**:35-43. DOI: 10.1016/j.jneuroim.2010.06.006

[80] Homma M, Uzawa A, Tanaka H, Kawaguchi N, Kanai T, Nakajima K, et al. A novel fusion protein, AChR-Fc, ameliorates myasthenia gravis by neutralizing antiacetylcholine receptor antibodies and suppressing acetylcholine receptor-reactive B cells. *Neurotherapeutics*. 2017;**14**:191-198. DOI: 10.1007/s13311-016-0476-9