Chapter

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

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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 α4β2 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 α1 as an example, we screened a PCR-generated mutant library of mouse nAChR α1 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 α1 ECD bound to a-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).

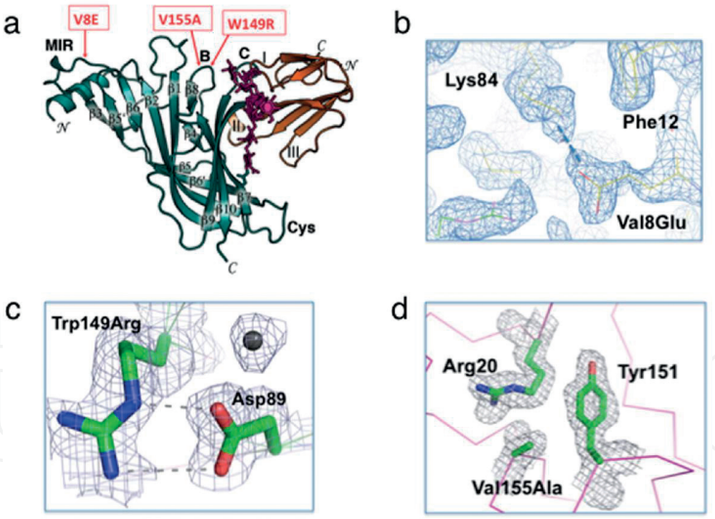

Figure 1.
Mutations that stabilize nAChR α1 ECD. (a) The three mutations (boxed and indicated by arrow) are mapped on the surface of nAChR α1 ECD (dark green) and away from the binding site of α-bungarotoxin (orange) and the glycan (magenta); (b) the mutation Val8Glu establishes a salt bridge with Lys84. 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 instrinsic 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 α1 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 α1, one β1, one δ, and one ε (adult form) or γ (fetal form) subunit(s) [18]. Extensive studies suggest that autoantibodies to α1 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 α1 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 α1 [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 α1 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 α1 in an upright orientation, away from the α-Btx (**Figure 2**). The Fab35 binding sites on nAChR α1 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 α1. Contacting residues from Fab35 and nAChR α1 (defined as being closer than 4.5 Å) can be mapped using the crystal structure. Such contacting analysis revealed several "hotspots" on nAChR α1 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 α1, two exposed residues, Arg6 and Asn10, which correspond to Arg6 and Lys10 in human nAChR α1, 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 Asp71of the MIR and Arg6 and Lys10 of the N-terminal helix, were indeed found to be interaction "hotspots" at the Fab35/nAChR α1 interface. More recent studies using natively folded nAChR α1α7 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**).

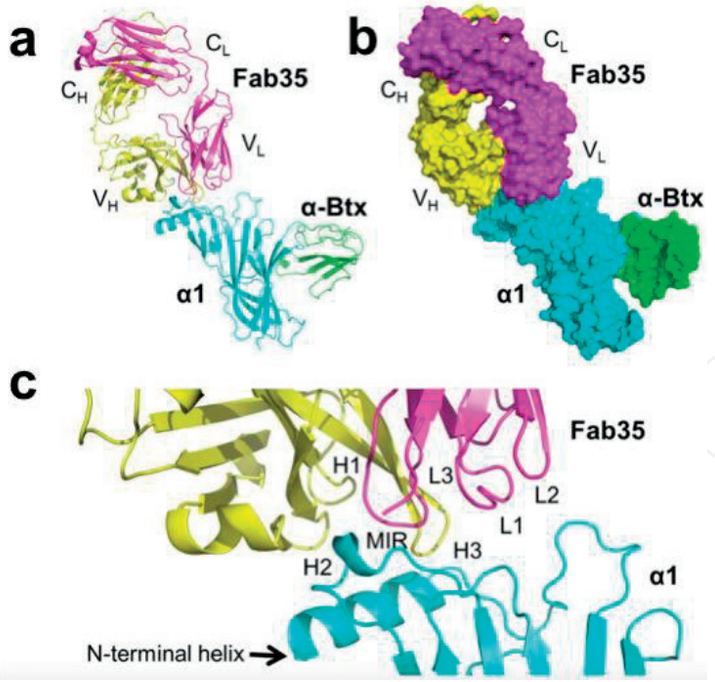

Figure 2.
Crystal structure of the ternary complex of nAChR α1 ECD bound by Fab35 and α-Btx. (a) Ribbon representation of nAChR α1 ECD (α1: cyan) in complex with α-Btx (green) and Fab35 (heavy chain (H, yellow) and light chain (L, magenta)). The variable domains (VH and VL) and the constant domains (CH and CL) 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]).

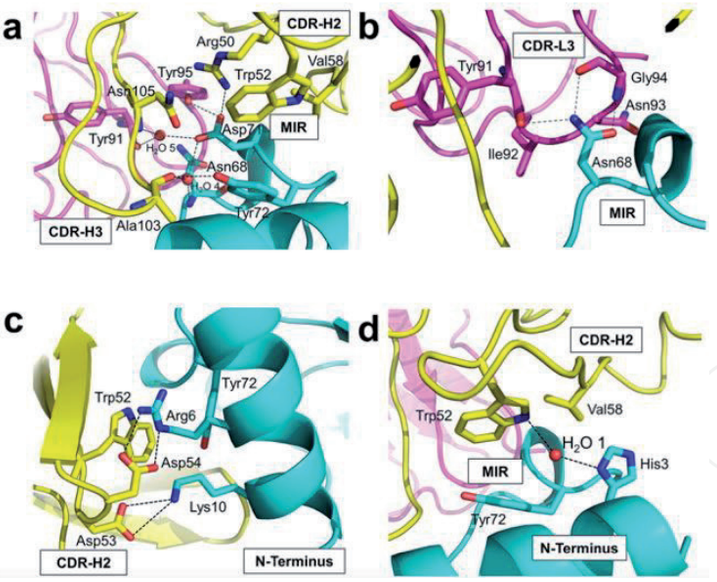

Figure 3.
Detailed interactions between Fab35 and nAChR α1 ECD at the binding interface. (a) Binding interactions at the vicinity of Asp71 of α1 (located at the MIR). (b) Interactions at the vicinity of Asn68 of α1 (located at the MIR). (c) Interactions involving Arg6 and Lys10 of α1 (located at the N-terminus of α1). (d) Interactions mediated by His3 of α1 (located at the N-terminus of α1) (Adapted from Noridomi et al. [58]).

Although biochemical mapping of antibody-binding residues on nAChR α1 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 predicated 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 α1-binding residues in Fab35 are also conserved in Fab198 and they appear to have similar contacts to nAChR α1 in the modeled Fab198/nAChR α1 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 α1, 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 α1-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 α1, confirming and extending the differences previously recognized between the two [52].

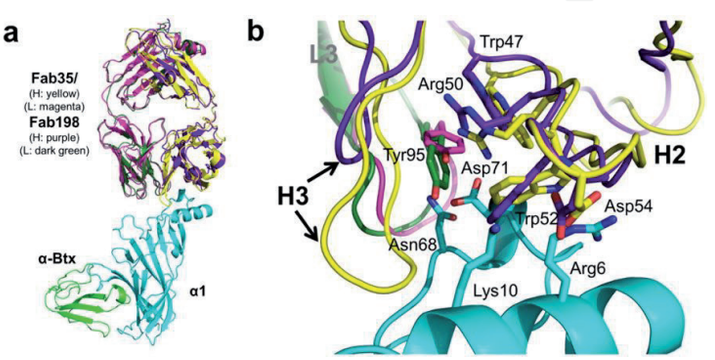

Figure 4.
Structural comparisons among MG mAbs. (a) Superposition of Fab198 [55] (heavy chain: purple and light chain: dark green) onto Fab35 in the Fab35/nAChR α1/α-Btx ternary complex using the Cα backbone. (b) Detailed comparison of the binding interface. The residues are colored according to their 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 α1 subunit did show significant correlation with disease severity [47]. These observations suggest that autoantibodies directed to nAChR α1 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 (α1, 67–76) and the N-terminal helix (α1, 2–14) (N-helix) and surrounding regions (α1, 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 α1 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

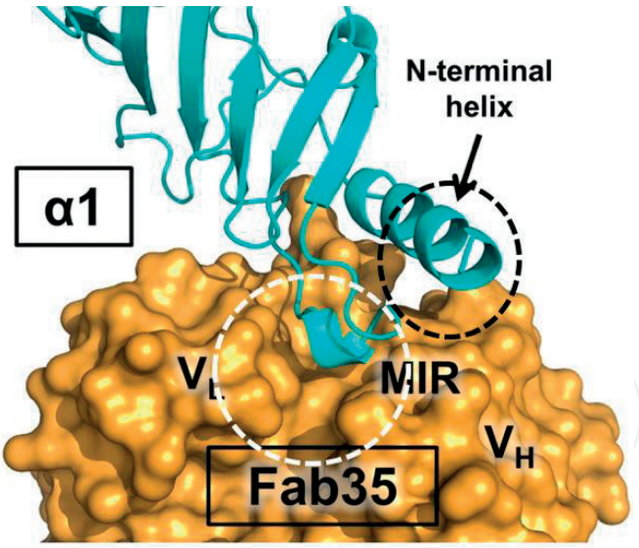

Figure 5.
Surface pockets on Fab35 bound by the nAChR MIR loop (white dashed circle) and the N-terminal helix (black dashed circle).

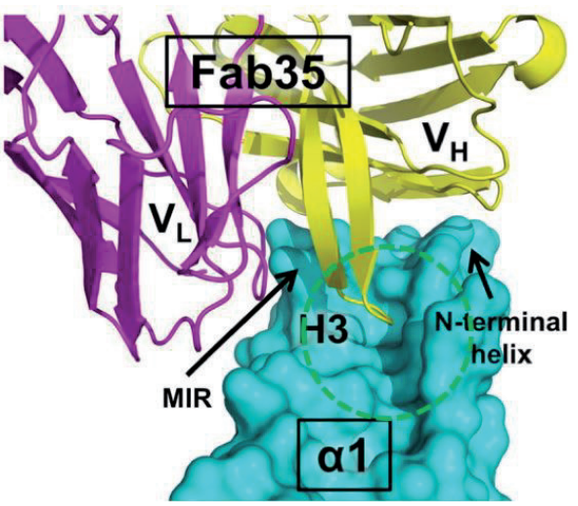

Figure 6.
The surface pocket (green dashed circle) on nAChR α1 bound by the CDR-H3 loop from Fab35 (indicated as H3 in the figure).

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 α1 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 α1 ECD to a plant toxin and showed its effectiveness in specifically killing of α1-specific B cells [78]. More recently, researchers have developed a variant of such strategy in which nAChR α1 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 FcRγIIB, which transduce negative signal for B cell activation. Consequently, such chimeric protein will specifically target the nAChR α1 specific B cell via the binding to the BCR and deliver negative signal to inhibit α1 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 α1 is just one subunit of the nAChR pentamer and is intrinsically unstable, making the expression of wild type nAChR α1 ECD in stable soluble form very challenging. However, as discussed earlier in this chapter, crystallography studies of nAChR α1 ECD in recent years have accumulated extensive experience and knowledge in designing strategic mutations to improve the stability and expression level of nAChR α1 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.

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