Diverse Fab specific for acetylcholine receptor epitopes from a myasthenia gravis thymus combinatorial library

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Abstract
The muscle weakness in myasthenia gravis (MG) is caused by heterogeneous high-affinity IgG autoantibodies to the nicotinic acetylcholine receptor (AChR), a complex ion channel glycoprotein. These antibodies are clearly responsible for reducing AChR numbers at the neuromuscular junction in myasthenia; however, the origins, diversity, specificity and pathogenicity of individual antibodies have not yet been established. We have cloned and characterized four different AChR-specific Fab from an MG patient’s thymus by screening an IgG1/κ gene combinatorial lambda phage library with soluble human AChR labeled with [125I]α-bungarotoxin. Unlike most previously cloned human antibodies, all four Fab immunoprecipitated soluble human muscle AChR. Two Fab strongly inhibited binding of mAb to the main immunogenic region on the α subunits and one Fab bound to an epitope on the fetal-specific γ subunit. In sensitivity and fine specificity, these Fab resembled the anti-AChR antibodies found in many MG patients, including the donor. The closest germline counterparts for their heavy chains were in VH families 1, 3 and 4; however, there were many differences consistent with an antigen-driven response of diverse B cell clones. The combinatorial approach holds promise for further analysis of human autoantibodies.

Introduction
Myasthenia gravis (MG) is an organ-specific autoimmune disease in which pathogenic IgG autoantibodies to the nicotinic acetylcholine receptor (AChR) lead to receptor loss and muscle weakness (reviewed in 1–3). The AChR is a pentameric transmembrane glycoprotein ion channel, composed of α2βγδ (fetal) or α2βδε (adult) subunits (4). Each subunit has a large, highly conformational extracellular domain at the N-terminus and four transmembrane regions. The α subunits bind ACh and the snake toxin α-bungarotoxin (α-BuTx). In MG, the autoantibodies are measured by immunoprecipitation of detergent-extracted human AChR labeled with [125I]α-BuTx. They are of high avidity (Kd <10−10 M), and heterogeneous in IgG subclass and light chain isotype, although IgG1 and κ often predominate (5). Variable proportions of the antibodies in different patients are directed against the ‘main immunogenic region’ (MIR) and the α-BuTx-binding sites on the extracellular aspects of the α subunits; some distinguish between fetal and adult AChR or show cross-reactivity with AChR from non-primate species (5–9).

Despite their clear involvement in disease pathogenesis, little is known of the V region genes that encode the H and L chains of these antibodies, mainly because of the difficulty in cloning human antibodies by conventional hybridoma technology (reviewed in 10). A few human monoclonal anti-AChR antibodies have been generated from hybridomas (11–13), but none has been shown to immunoprecipitate detergent-extracted human AChR and some bound equally to Torpedo AChR, a specificity which is uncommon in MG sera (1,8), questioning their relevance to the pathogenic autoantibodies.

There are now a number of different systems for cloning and expressing Ig by recombinant techniques (reviewed in 14–16). We have constructed an Ig gene combinatorial library
and expressed it using the Immunozap vector (as reviewed in 16). We used the thymus of a myasthenic patient, removed for therapeutic reasons, as a source of AChR-specific plasma cell mRNA, because the thymus gland in young MG patients often shows lymphofollicular hyperplasia and is rich in plasma cells that spontaneously synthesize AChR autoantibodies with specificities similar to those in the serum (2,3,17–20). We screened this library with soluble 125I-α-BuTx-labeled AChR, and isolated and characterized four unrelated Fab.

Methods

Patient selection and combinatorial Ig gene library construction

The donor was a 22-year-old female patient (AB) with a 4-year history of MG (grade IIA) and a high serum anti-AChR titer (500 nmol/l, i.e. ~50 pmol/mg of IgG). The thymus showed only mild hyperplasia, but cell suspensions prepared with collagenase and dispase (20) spontaneously synthesized high levels of anti-AChR antibodies with low levels of total IgG (471 fmol/0.38 µg/10^6 cells, i.e. ~1200 pmol/mg of IgG) suggesting that it is highly enriched for AChR-specific plasma cells. From these cells we prepared mRNA (Quickprep Kit; Pharmacia Biotech, Piscataway, NJ) for reverse transcription of cDNA (First Strand Synthesis Kit; Stratagene, La Jolla, CA). Ig H chain DNA was amplified by the PCR (21) using a panel of sense oligonucleotide primers designed to amplify V_{H}1–V_{H}6 families as in (22) and an anti-sense primer corresponding to the 3’ end of the IgG C_{H}1 domain (23). Similarly, a panel of sense oligonucleotide primers, designed to cross-hybridize with all V_{κ} gene families (24), and an antisense primer to the 3’ end of the κ L chain (22) were used in L chain DNA amplification. The primers incorporated restriction sites for cloning and the PCR was performed as previously described (22). PCR products were digested with XhoI–SpeI for the H chain and SacI–XbaI for the L chain, and ligated into the same sites in the Immunozap H and L bacteriophage λ vectors (Stratagene). The unamplified H and L chain libraries each contained ~5×10^6 recombinants. Subsequently, the H chain DNA (digested with HindIII and EcoRI) was ligated with κ chain DNA (digested with MluI and EcoRI) to yield a combinatorial library of 7.23×10^6 recombinants.

Screening for AChR binding Fab and Fab expression

The unamplified AB IgG1/κ combinatorial library was screened in XL1-Blue cells in conventional filter lift assays (25) using human muscle ACHR labeled with 2 nM 125I-α-BuTx (>2000 Ci/mmoll; Amersham International, Amersham, UK). Positive plaques were identified by autoradiography and cloned to homogeneity. Plasmids (BlueScript SK) were excised from the Immunozap vector using the helper phage R408 (Stratagene), and nucleotide sequencing of the Fab H and L chain inserts was performed in both directions by the dideoxynucleotide chain termination method (26).

Fab were expressed as soluble proteins in XL1-Blue cells, as previously described (27). Briefly, protein synthesis was induced with 1 mM isopropyl-thio-galacto-pyranoside (Sigma, St Louis, MO) for 1 h at 37°C. The cells were then pelleted, frozen at –20°C, and resuspended in 0.02 volumes of 10 mM Tris, pH 8.0, containing 2 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin and 0.1 mM phenylmethylsulfonyl fluoride (all from Sigma). The suspension was sonicated, membranes pelletted by centrifugation at 4000 g and the Fab protein was concentrated from the supernatant using a Protein G–Sepharose column (Pierce, UK).

Immunoprecipitation of 125I-α-BuTx–AChR

AB serum and purified Fab were tested by immunoprecipitation for binding to AChR from human, rat and mouse muscle, from wild-type TE671 cells that express fetal-type AChR, from TE671-c transfectants that express mainly adult-type AChR (28), from Xenopus oocytes expressing AChR subunits (29), and from Torpedo electric organ. In each case, AChR was extracted in Triton X-100 and labeled with 2–3 nM 125I-α-BuTx (Amersham International; 220 Ci/mmoll), as previously described (5,30). The total amount of 125I-α-BuTx–AChR in each extract was determined by DE81 filter disc assay or by immunoprecipitation (see below) with a high titer, species cross-reactive MG serum (5,30).

Serial dilutions of AB serum or 10–100 µl of each Fab were added to each 125I-α-BuTx–AChR preparation (~30 fmol in 50 µl total volume) and incubated for 2 h at room temperature or overnight at 4°C. Subsequently, IgG–AChR or Fab–AChR complexes were precipitated by addition of 1 µl healthy control serum (as carrier IgG, if necessary) and 15–20 µl of a goat anti-human IgG (Lawrence Laboratories, Queensland, Australia), that efficiently precipitated both IgG and Fab. Serum and Fab were serially diluted for testing against human, mouse and rat muscle AChR, and were tested undiluted against Torpedo AChR. Results of precipitation by the appropriate amount of healthy control serum or a Fab control were subtracted.

Epitope specificity of AB serum and Fab

To investigate the epitopes bound by AB serum, AChR was preincubated with mAb that bind to different epitopes on human muscle AChR (30) and inhibition of AB serum binding analyzed as previously described (8,31). Aliquots of 50 µl of 125I-α-BuTx–AChR were incubated overnight with each mAb at 1 µl of ascites/ml, and 0.1 µl of AB serum was added for 2 h at room temperature, followed by 15 µl goat anti-human IgG (pre-absorbed with 20% normal mouse serum to ensure that it did not immunoprecipitate the mAb) and 1 µl normal human serum (to act as carrier IgG). Results were expressed as percent inhibition: (c.p.m. precipitated by AB serum in the absence of mAb – c.p.m. precipitated by AB serum in the presence of mAb)/(c.p.m. precipitated by AB serum in the absence of mAb – c.p.m. precipitated by healthy control serum).

For Fab, the opposite approach was easier because our goat anti-mouse IgG did not precipitate Fab–AChR complexes. Aliquots of 50 µl of each Fab were preincubated overnight with 50 µl 125I-α-BuTx–human AChR, and then 0.1 µl of each mAb was added and the incubation continued for 2–4 h at room temperature. mAb were then immunoprecipitated with 1 µl of normal mouse serum as carrier and 15 µl goat anti-mouse IgG (The Binding Site, Birmingham, UK). Inhibition of mAb binding by each Fab is expressed as: 100×(c.p.m. precipitated by mAb in the absence of Fab –
Results

Ig genes encoding AChR-specific Fab

Five cloned Fab (called AB1–5) were initially identified in the thymic IgG1/k Ig gene combinatorial library (expressed in bacteriophage λ) by binding of [125I]-α-BuTx–AChR to plaque nitrocellulose lifts. Both H and L chain variable sequences of subunit of the AChR that is characteristic of fetal or denervated muscle. We therefore tested the serum and Fab for reactivity with AChR extracted from TE671–AB1 and 2 proved to be completely identical (now called muscle. We therefore tested the serum and Fab for reactivity with AChR.

Characterization of AB serum and Fab

All Fab immunoprecipitated soluble [125I]-α-BuTx–labeled AChR from human muscle extracts (that contain both adult- and fetal-type AChR) in a dose-dependent manner. However, as expected, the apparent titers of active Fab in the concentrated bacterial lysates were much lower than that of the polyclonal IgG autoantibodies in AB's serum (Fig. 2a). AB's serum showed considerable cross-reactivity with mouse and rat AChR (Fig. 2b) but did not bind appreciably to Torpedo AChR. Fab AB1/2 precipitated both mouse and rat AChR relatively well (but not Torpedo AChR), whereas AB3 greatly preferred mouse AChR. AB4 and 5 bound much less to either (Fig. 2b). Neither the Fab nor serum from AB inhibited binding of [125I]-α-BuTx to human AChR or inhibited the function of dispersion of hyperplastic thymus (20). For screening the extracellular domain of the α subunit (8). These specificity has recently been demonstrated by Western blotting on recombinant human AChR subunits (L. Jacobson et al., in preparation). These mAb compete variably with the autoantibodies in MG patients' sera and do not interfere with binding of α-BuTx (8,11). Between 50 and 75% of the anti-AChR antibody in AB's serum was inhibited by preincubation with mAb D6α or C3α (Fig. 3a). In addition ~50% of AB's serum antibody was inhibited by mAb C2γ or B8γ. Little or no inhibition was found with mAb B3β. We found a range of specificities in the Fab. Fab AB1/2 was evidently MIR-specific, inhibiting the binding of D6α and C3α [Fig. 3a; and also anti-MIR mAb 35 (6), not shown], while showing no inhibition of B3β, C2γ or B8γ. Fab AB1/2 slightly enhanced, rather than inhibited, the binding of B3β as is often found with MG serum antibodies (8). Fab AB3 showed a similar profile (Fig. 2a). Fab AB4 showed less clear specificity, but AB5 differed from the others in competing with mAb B3β, and especially with both γ-specific mAb, C2γ and B8γ.

Discussion

We have cloned four different AChR-specific autoantibodies from an Ig gene combinatorial library constructed from a MG patient's thymus and expressed them as Fab. Despite their monovalency, the Fab immunoprecipitated solubilized human muscle AChR (concentration of AChR ~1 nM), suggesting the high affinity typical of MG serum antibodies (5), although further studies are required to confirm this point. Moreover, their fine specificities were similar to those found in the donor's serum in that two were directed against epitopes of the MIR and one bound to an extracellular epitope of the γ subunit. Hence these heterogeneous antibodies with their diverse variable gene usage and frequent apparent mutations in the heavy chains are likely to reflect those expressed in vivo.

To clone the Fab we took advantage of the enrichment of plasma cells, with their abundant mRNA, after enzymic dispersion of hyperplastic thymus (20). For screening plaques, we mimicked the immunoprecipitation assay for MG anti-AChR antibodies by using high specific activity [125I]-α-BuTx to label human AChR. As in most MG sera (5,9), all four Fabpreferentially recognized human AChR and the two tested did not bind Torpedo AChR. Some human mAb obtained from hybridomas by others have shown specificity for electric fish AChR against which they were initially screened (11–13) and did not immunoprecipitate human AChR in the standard assay. Such antibodies are clearly not typical of those present in MG sera.

Only two other groups have reported results with combinatorial libraries. Zeidel et al. (38) used the alternative filamentous phage display pComb3 system and selected four Fab with relatively low affinity for purified Torpedo AChR; however, these also bound to TE671 cells on FACS analysis, suggesting reactivity with human AChR. Very recently, Graus et al. (39) used pComb3H and selected Fab on mAb-immobilized human AChR. Out of 43 positive clones, derived from two MG donor thymuses, they found only four distinct Fab. More-
Acetylcholine receptor-specific Fab cloned from myasthenic thymus

Table 1. H and L chain genes encoding four anti-AChR Fab

<table>
<thead>
<tr>
<th></th>
<th>AB1/2</th>
<th>AB3</th>
<th>AB4</th>
<th>AB5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy chain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vh family</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>germline gene</td>
<td>DP42 (32)</td>
<td>DP65 (32)</td>
<td>21–1/3–1/DP7 (32,33)</td>
<td>DP58 (32)</td>
</tr>
<tr>
<td>total R:S</td>
<td>10:7</td>
<td>15:10</td>
<td>16:9</td>
<td>14:1</td>
</tr>
<tr>
<td>CDR1 R:S</td>
<td>2.0</td>
<td>3.2</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>CDR2 R:S</td>
<td>3.1</td>
<td>1.2</td>
<td>1.01</td>
<td>8.0</td>
</tr>
<tr>
<td>CDR3 region (nucleotides)</td>
<td>45</td>
<td>30</td>
<td>24</td>
<td>42</td>
</tr>
<tr>
<td>Jh region</td>
<td>Jh4B</td>
<td>Jh3A</td>
<td>Jh4B</td>
<td>Jh5B</td>
</tr>
<tr>
<td><strong>Light chain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vk family</td>
<td>III</td>
<td>I</td>
<td>III</td>
<td>III</td>
</tr>
<tr>
<td>germline gene</td>
<td>humv325/DPK22 (34,35)</td>
<td>O2/O12/DPK9 (35,36)</td>
<td>humv325/DPK22 (34,35)</td>
<td>L25/DPK23 (35,37)</td>
</tr>
<tr>
<td>total R:S</td>
<td>14:11</td>
<td>8:5</td>
<td>6:4</td>
<td>9:3</td>
</tr>
<tr>
<td>CDR1 R:S</td>
<td>5:2</td>
<td>1:2</td>
<td>1:0</td>
<td>2:0</td>
</tr>
<tr>
<td>CDR2 R:S</td>
<td>4:2</td>
<td>1:1</td>
<td>2:1</td>
<td>0:2</td>
</tr>
<tr>
<td>CDR3 region (nucleotides)</td>
<td>3:2</td>
<td>3:1</td>
<td>1:1</td>
<td>3:0</td>
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<tr>
<td>Jk region</td>
<td>Jk3</td>
<td>Jk3</td>
<td>Jk3</td>
<td>Jk4</td>
</tr>
</tbody>
</table>

R:S = replacement:silent mutations (total no. of substitutions resulting in codon changes:total no. of substitutions not resulting in codon changes).

**Fig. 1.** (a) Deduced amino acid sequences of the Vh and D regions encoding four AChR-specific Fab cloned from an IgG/κ combinatorial library constructed from thymic cells from a female MG patient (AB). The library was screened using 125I-α-BuTx-labeled human AChR. The Vh regions are compared with the amino acid sequences of their closest known germline genes. Amino acid identities are shown by dots; amino acid differences are indicated. (b) Deduced amino acid sequences of the Vk regions. The Vk regions are compared with the derived amino acid sequences of their closest germline genes. The sequences are available from EMBL/GenBank/DDBJ under accession nos Z84387 (AB1H), Z84388 (AB3H), Z84389 (AB4H), Z84390 (AB5H), Z84391 (AB1k), Z84392 (AB3k), Z84393 (AB4k) and Z84394 (AB5k).

However, the three from one donor had clonally related heavy chains and two of these also had related light chains (39). Since these Fab all bound to the MIR, phage display with selection on immobilized AChR may bias against other specificities. Conversely, our use of 125I-α-BuTx to label the AChR may bias against antibodies to the α-BuTx binding site. Nevertheless, the greater diversity obtained in our study of IgG1 Fab from a single thymus is striking and consistent with previous observations that suggested greater diversity after selection by plaque screening than by phage display.
Acetylcholine receptor-specific Fab cloned from myasthenic thymus

Fig. 2. (a) Binding of AB serum and Fab to AChR. 125I-α-BuTx-labeled human AChR was incubated with increasing amounts of serum (1:100 diluted) or Fab. Immunoprecipitation was performed by addition of goat anti-human Ig with 1 µl normal human serum as carrier. Results shown are from a single experiment on one batch of Fab preparations. (b) Cross-reactivity of AB serum and Fab with rat and mouse AChR. Results in each case are expressed as percentage of the reactivity with human AChR, based on titrations of each Fab against human, rat and mouse AChR [e.g. as in (a)]. Reactivity with Torpedo AChR was tested with serum at 1:50 and undiluted Fab1/2.  

It will clearly be important to compare further the results of the two methods in MG and other autoimmune conditions.

One criticism of any combinatorial approach is that the Fab H/L chain pairings obtained do not necessarily represent those found in vivo. It is therefore important to test whether the Fab have similar specificities to serum antibodies. Several of the features that we observed in Fab AB1–5 mirrored those that are seen in MG. MG anti-AChR antibodies often show preference for fetal- rather than adult-type AChR, react variably with AChR from other species, and are heterogeneous in their fine specificity for the MIR and other epitopes as demonstrated by competition with mAb (5–9,31). Three of our Fab did not distinguish between fetal- and adult-type AChR, but AB5 showed a marked preference for fetal-type AChR, was able to inhibit binding of γ subunit-specific mAb and immunoprecipitated γγ dimers but not ω dimers. Differences in reactivity with mouse or rat AChR were clearly demonstrated by Fab1/2 and 3, even though they each showed specificity for the MIR which is well conserved between species (7). These specificities were represented in AB serum which showed strong cross-reactivity with mouse and rat AChR, and inhibition by the MIR- and γ subunit-specific mAb. We did not directly test the ability of the Fab to prevent binding of AB’s serum antibody, but the inhibition studies with mAb indicate that they bound to the same or closely overlapping epitopes. Thus, we feel confident that the fine specificities of the Fab for extracellular AChR epitopes reflect those of donor AB.

Anti-AChR antibodies in MG reduce numbers of functional AChR by three main mechanisms (40). Antibodies to the ACh/α-BuTx-binding sites are a minority in most patients but can be especially pathogenic (41). However, none of the Fab caused inhibition of α-BuTx binding, an activity that would have complicated their initial detection and was not evident in the donor’s serum. The two more common pathogenetic mechanisms are complement-mediated damage and accelerated degradation resulting from cross-linking and internalization of the AChR. Since our primer preferentially amplifies IgG1 sequences, the Fab must derive from a complement-activating subclass. Moreover, antibodies with MIR specificity are particularly efficient at modulating AChR from cell surfaces (42); thus the parent IgG of the MIR-reactive Fab (AB1/2 and AB3) might combine these two pathogenic properties.

A key question concerns the initiating stimulus to the autoanti-AChR response. Many patients, especially with early-onset MG, have some anti-AChR antibodies that bind preferentially to fetal rather than adult AChR (5). Their prevalence suggests a role for myoid cells in the thymus that express AChR of this isoform (43). Since the thymus usually also shows hyperplasia with germinal centers and contains plasma cells autonomously secreting anti-AChR antibodies (2,17,20), it may well be both a site of autosensitization and a source of autoanti-AChR. Fab have now been cloned by three groups (Table 2). The H chains of these heterogeneous Fab apparently derive from four different V H genes in three families. That already argues strongly against the oligoclonal origin for these autoantibodies that has been suggested for some human autoimmune conditions (44,45) and shown for DNA autoantibodies in a mouse model of systemic lupus erythematosus (46). Anti-AChR Fab have now been cloned by three groups (Table 2). None of the germline genes is the same, but those previously reported are from the V γ1 and V γ3 families. Fab AB3 is the first to use V H4 genes, although they were abundantly expressed in normal human thymus (47). The V H gene DP42 encoding Fab AB1/2 is a polymorphic variant of B-1B, the putative germline origin of some thyroid-peroxidase-specific Fab (27). Furthermore, DP65 is the germline origin of both AChR-specific Fab AB3 and a thyroglobulin-specific mAb (48). Two groups of V H genes utilized by the AB Fab are abundant in the expressed repertoire, O2/D012 and A27/humkv325/DPK22, and have been noted in other organ-specific autoantibodies; the former encodes numerous Fab specific for thyroid peroxidase (reviewed in 50) and the latter encodes a human mAb to thyroglobulin (48).

Finally, the heavy chains, in particular, showed many differences from the closest germline sequences; in AB4 and AB5, they tended to cluster in or near the CDR2s, as also noted...
Fig. 3. Epitope specificity of AB serum and Fab. (a) Competition between AB serum or Fab with mAb to extracellular epitopes on AChR subunits. In each case, the results are presented as percentage of the total anti-AChR activity (see Methods for details of the calculations). Error bars are SDs of two to four determinations using different batches of Fab preparations. (b) Specificity of Fab5 for fetal-type AChR. Immunoprecipitation of $^{125}\text{I-}\alpha$-BuTx-labeled AChR from TE671-ε (adult-type) or TE671-γ (fetal-type) by serum and Fab 1 and 5, and of $^{125}\text{I-}\alpha$-BuTx-labeled αγ and αε dimers by Fab. The dimers were expressed in Xenopus oocytes by injection with cRNA, and extracted and labeled with $^{125}\text{I-}\alpha$-BuTx as described in (28).

Table 2. $V_H$ genes encoding human AChR Fab from combinatorial libraries

<table>
<thead>
<tr>
<th>Source, system</th>
<th>Name</th>
<th>$V_H$ (closest germline)</th>
<th>$V_L$ (closest germline)</th>
<th>Selection/specitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL, pComb3</td>
<td>$V_H1$ L33</td>
<td>not given</td>
<td></td>
<td>selected on Torpedo AChR; binding to TE671 demonstrated by FACS</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>$V_H1$ (DP-15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$V_H3$ (Vc26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$V_H3$ (DP-51)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus, pComb3</td>
<td>66</td>
<td>$V_H3$ (DP47)</td>
<td>$V_L3$ (DPK22)</td>
<td>selected on immobilized AChR; immunoprecipitated human AChR</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>$V_H3$ (DP47)</td>
<td>$V_L3$ (DPK22)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>587</td>
<td>$V_H3$ (DP47)</td>
<td>$V_L3$ (DPK9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>637</td>
<td>$V_H3$ (Vc3–8)</td>
<td>$V_L$ (IgL6/S1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus, Immunozap</td>
<td>AB1/2</td>
<td>$V_H3$ (DP-42)</td>
<td>$V_L3$ (DPK22)</td>
<td>selected by binding to $^{125}\text{I-}\alpha$-BuTx–AChR on plaque</td>
<td>present study</td>
</tr>
<tr>
<td></td>
<td>AB3</td>
<td>$V_H4$ (DP-65)</td>
<td>$V_L3$ (DPK9)</td>
<td>lifts; immunoprecipitated human AChR</td>
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</tr>
<tr>
<td></td>
<td>AB4</td>
<td>$V_H1$ (DP-7)</td>
<td>$V_L3$ (DPK22)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>AB5</td>
<td>$V_H3$ (DP-58)</td>
<td>$V_L3$ (DPK23)</td>
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by Graus et al. (39) for their Fab. The ratio of replacement to silent mutations in these CDR2s is similar to those in other human mAb, as discussed by Chang and Casali (51). There was a similar tendency in the L chain of AB1/2, though less so in the others. We found no obvious recurring motifs or convergent mutations, even when comparing with the equally
diverse mouse anti-AChR mAb sequences (49). These findings are consistent with an antigen-driven diversification of the repertoire, reflecting mutations accumulating in germinal center B cells. The resulting heterogeneity in epitope specificities may well enhance complement activation at the endplate by allowing denser packing of Fc pieces. However, it is a particularly intriguing possibility that some antibody species might interfere in pathogenicity and thus play a protective role, perhaps helping to explain the poor correlation between antibody titer and disease severity (1–3, 40). The availability of cloned human anti-AChR antibodies, representative of the repertoire in the patient’s serum, should allow this possibility to be investigated. If confirmed, e.g., by transfer to other species using reconstructed IgG, that might open exciting new therapeutic avenues.

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Abbreviations

\[ \alpha -\text{BuTx} \quad \alpha -\text{bungarotoxin} \]

AChR  acetylcholine receptor

CDR  complementarity determining region

MG  myasthenia gravis

MIR  main immunogenic region

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Acetylcholine receptor-specific Fab cloned from myasthenic thymus


