

Heterogeneity of Acetylcholine Receptor Autoantibody–Mediated Complement Activity in Patients With Myasthenia Gravis

Abeer H. Obaid, BS, Chryssa Zografou, PhD, Douangson D. Vadysirisack, PhD, Bailey Munro-Sheldon, PhD, MS, Miriam L. Fichtner, MD, Bhaskar Roy, MBBS, MHS, William M. Philbrick, PhD, Jeffrey L. Bennett, MD, PhD, Richard J. Nowak, MD, MS, and Kevin C. O'Connor, PhD

Correspondence
Dr. O'Connor
kevin.oconnor@yale.edu

Neurol Neuroimmunol Neuroinflamm 2022;9:e1169. doi:10.1212/NXI.0000000000001169

Abstract

Background and Objectives

Autoantibodies targeting the acetylcholine receptor (AChR), found in patients with myasthenia gravis (MG), mediate pathology through 3 mechanisms: complement-directed tissue damage, blocking of the acetylcholine binding site, and internalization of the AChR. Clinical assays, used to diagnose and monitor patients, measure only autoantibody binding. Consequently, they are limited in providing association with disease burden, understanding of mechanistic heterogeneity, and monitoring therapeutic response. The objective of this study was to develop a cell-based assay that measures AChR autoantibody–mediated complement membrane attack complex (MAC) formation.

Methods

An HEK293T cell line—modified using CRISPR/Cas9 genome editing to disrupt expression of the complement regulator genes (CD46, CD55, and CD59)—was used to measure AChR autoantibody–mediated MAC formation through flow cytometry.

Results

Serum samples ($n = 155$) from 96 clinically confirmed AChR MG patients, representing a wide range of disease burden and autoantibody titer, were tested along with 32 healthy donor (HD) samples. AChR autoantibodies were detected in 139 of the 155 (89.7%) MG samples through a cell-based assay. Of the 139 AChR-positive samples, autoantibody-mediated MAC formation was detected in 83 (59.7%), whereas MAC formation was undetectable in the HD group or AChR-positive samples with low autoantibody levels. MAC formation was positively associated with autoantibody binding in most patient samples; ratios (mean fluorescence intensity) of MAC formation to AChR autoantibody binding ranged between 0.27 and 48, with a median of 0.79 and an interquartile range of 0.43 (0.58–1.1). However, the distribution of ratios was asymmetric and included extreme values; 16 samples were beyond the 10–90 percentile, with high MAC to low AChR autoantibody binding ratio or the reverse. Correlation between MAC formation and clinical disease scores suggested a modest positive association ($\rho = 0.34$, $p = 0.0023$), which included a subset of outliers that did not follow this pattern. MAC formation did not associate with exposure to immunotherapy, thymectomy, or MG subtypes defined by age-of-onset.

Discussion

A novel assay for evaluating AChR autoantibody–mediated complement activity was developed. A subset of patients that lacks association between MAC formation and autoantibody binding or

From the Department of Neurology (A.H.O., C.Z., B.M.-S., M.L.F., B.R., R.J.N., K.C.O.C.), Yale School of Medicine, New Haven, CT; Department of Immunobiology (A.H.O., C.Z., M.L.F., W.M.P., K.C.O.C.), Yale School of Medicine, New Haven, CT; Institute of Biomedical Studies (A.H.O.), Baylor University, Waco, TX; UCB Pharma (D.D.V.), Cambridge, MA; and Departments of Neurology and Ophthalmology (J.L.B.), Programs in Neuroscience and Immunology, University of Colorado Anschutz Medical Campus, Aurora.

Go to [Neurology.org/NN](https://www.neurology.org/NN) for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Glossary

AChR = acetylcholine receptor; **CBA** = cell-based assay; **CHO** = Chinese hamster ovary; **EOMG** = early-onset MG; **FACS** = fluorescence-activated cell sorting; **HD** = healthy donors; **HEK** = human embryonic kidney; **LOMG** = late-onset MG; **MAC** = membrane attack complex; **mAbs** = monoclonal antibodies; **MFI** = mean fluorescence intensity; **MG** = myasthenia gravis; **MGC** = MG composite score; **MGFA** = Myasthenia Gravis Foundation of American; **MMN** = multifocal motor neuropathy; **NHS** = normal human serum; **NMJ** = neuromuscular junction; **NMOSD** = neuromyelitis optica spectrum disorder; **WT** = Wild type.

disease burden was identified. The assay may provide a better understanding of the heterogeneous autoantibody molecular pathology and identify patients expected to benefit from complement inhibitor therapy.

The impairment in autoimmune myasthenia gravis (MG) is caused by autoantibodies that target components of the neuromuscular junction (NMJ).¹⁻³ The most common MG subtype is characterized by autoantibodies targeting the acetylcholine receptor (AChR).¹ Detection of AChR autoantibodies confirms a diagnosis of MG; however, titers (serum concentration) vary widely among patients and within individuals during their disease prevalence, and it is generally recognized that cross-sectional measurements of titer do not correlate well with disease severity among different patients.⁴⁻⁷

AChR autoantibodies are broadly polyclonal and heterogeneous in their specificity; MG serum can include autoantibodies that can recognize any of the 4 different AChR subunits (α , β , ϵ/γ , or δ).⁸ AChR autoantibodies can use 3 distinct mechanisms⁹⁻¹⁵ to effect pathology: (1) complement-directed tissue damage, (2) blocking the binding site for acetylcholine on the receptor, and (3) modulation (internalization) of the AChR. Furthermore, there may also be a subgroup of binding-only antibodies that recognize AChR in clinical assays but do not have any pathogenic properties.

In addition to the lack of understanding concerning the disconnect between autoantibody titer and disease severity, gaps in our knowledge have recently become more consequential because new therapeutics specifically targeting the pathogenic mechanisms of AChR autoantibodies—including complement inhibitors—have been introduced.¹⁶ Eculizumab is a recently approved biological therapeutic for the treatment of generalized AChR MG¹⁷; it inhibits autoantibody-mediated activity of the terminal complement cascade. The phase III clinical trial showed efficacy, but approximately 40% of AChR autoantibody-positive patients did not meet the trial endpoint without longer-term treatment, and some experienced exacerbation events requiring rescue therapy.¹⁷⁻¹⁹ These data are challenging to reconcile because the poor responders had measurable circulating AChR autoantibodies. This trial also highlights the limitations of AChR autoantibody titer as a biomarker and, importantly, underscores the need for further understanding of AChR autoantibody-mediated pathogenic mechanisms so that response to treatments can be better predicted. To that end, we sought to develop an *in vitro* assay that measures AChR autoantibody-mediated complement activation.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by Yale University's Institutional Review Board (clinicaltrials.gov NCT03792659). Informed written consent was received from all participating patients before inclusion in this study. Serum samples were collected from AChR MG patients at the Yale Medicine Myasthenia Gravis Clinic.²⁰ Demographics and clinical data are summarized in Table e1.

CRISPR/Cas9 Genome Editing of HEK293T Cells

CD46, CD55, and CD59 CRISPR sgRNAs (Table e2) were cloned into the pSpCas9(BB)-2A-Puro (PX459) V2.0²¹ vector (a gift from Dr. Feng Zhang [Addgene plasmid # 62,988]) and transfected into human embryonic kidney (HEK) 293T cells (ATCC CRL3216). The cells were harvested after 48 hours, and dilutions were used to derive single-cell clonal cell lines. These clones were then tested phenotypically for the deletions of CD46, CD55, and CD59 through PCR, Sanger sequencing, and flow cytometry. Clones that exhibited deletions underwent fluorescence-activated cell sorting (FACS) to ensure the selection of cells with the 3 deletions.

Autoantibody Binding Live Cell-Based Assay

Wild type (WT), triple KO HEK293T, or Chinese hamster ovary (CHO) cells-61 were transfected with adult AChR (2α , β , δ , and ϵ) and rapsyn-GFP plasmids (generous gifts from Drs. Angela Vincent, David Beeson, and Patrick Waters) using branched polyethylenimine or Lipofectamine 2000 (CHO only). After a 24-hour incubation, the medium was refreshed, and the cell-based assay (CBA) was conducted the following day.

Cells were exposed to serum (1:20 dilution) from healthy donors (HDs) or MG patients or monoclonal antibodies (mAbs) (10 $\mu\text{g}/\text{mL}$). Bound antibodies were detected with an anti-human IgG Fc γ (309-605-008; Jackson ImmunoResearch) with a LSR Fortessa (BD Biosciences). FlowJo software (v10.6.2) was used for analysis. In serial dilution testing, some serum samples can provide aberrantly low CBA

Δ mean fluorescence intensity (MFI) values when tested at 1:20 because of assay saturation and/or interference.

Accordingly, these data points were not considered in the analyses. For testing AQP4 binding, cells were transiently transfected with an AQP4-GFP (M1 isoform) plasmid²² using the CBA conditions described earlier.

Complement CBA

Complement-competent normal human serum (NHS) and factor B–depleted human serum were both purchased from Complement Technologies (Tyler, TX). The same lot was used within experiments. Human recombinant mAbs were expressed as previously described.²³

CD46, CD55, CD59 KO HEK293T cells or CHO cells were prepared as outlined in the live CBA. Cells were washed twice and resuspended in complete DMEM. Cells were exposed to AChR MG patient serum, HD serum (both heat-inactivated [HI]), or control mAbs suspended in 25% NHS or factor B–depleted serum (B-dHS). Membrane attack complex (MAC) formation was allowed to proceed for 3 hours at 37°C and fixed with 2% paraformaldehyde at 4°C for 15 minutes. The cells were then stained with anti-C9 neoantigen (Hycult, Cat# HM2264), followed by washing and secondary staining (APC [Cat # 407109] or PE IgG2a clone RMG2a-62 [Cat# 407109]). FACS analysis was conducted as described for the binding CBA.

Statistical Analysis

Threshold values for the CBA and complement CBA positivity were determined by the application of a cutoff value, determined by calculating the mean +4 SD of HD subject samples. For descriptive statistics, mean, median, or percentage were used as appropriate. A comparison between 2 groups was performed using the Mann-Whitney *U* test, and the Spearman correlation was used to examine the relationship between 2 parameters. A box-whisker plot (whisker representing 10–90 percentile) was used to graphically represent the distribution of the ratios to examine the relationship between MAC and AChR autoantibody binding. *p*-values below 0.05 were considered statistically significant. All statistical analyses were performed on Graphpad Prism software (v9.1.2). STROBE cohort reporting guidelines were used.²⁴

Data Availability

Anonymized data will be shared on request from qualified investigators and completion of materials transfer agreements.

Results

Measuring Autoantibody-Mediated MAC Formation Using a CBA Platform

AChR autoantibodies can be measured with live CBAs.²⁵ In this assay format, recombinant human AChR subunits and the clustering protein, rapsyn, were expressed in HEK cells and

tested for binding of autoantibodies. We sought to measure AChR autoantibody–mediated complement activity by modifying the clustered AChR CBA. To that end, the assay was performed by using autoantibodies (recombinant mAbs or autoimmune patient–derived serum, in which the complement was heat-inactivated) and NHS as a consistent complement source.

We first evaluated antibody-dependent MAC formation, by detecting a C9 neo-epitope, with the modified CBA using an established positive control; specifically, human aquaporin (AQP4) autoantibodies from patients with neuromyelitis optica spectrum disorder (NMOSD), which have been demonstrated to efficiently fix complement *in vitro*.²⁶ AQP4 was expressed in HEK293T cells, and a human AQP4-specific recombinant IgG1 subclass mAb (mAb-58²⁷) or serum from an AQP4 autoantibody–positive NMOSD patient was added along with NHS. MAC formation was detected when the AQP4-specific mAb or NMOSD serum and NHS were present. MAC formation was not detected in the absence of an AQP4-specific antibody source, absence of NHS, or when heat-inactivated NHS was included (eFigure 1A, eFigure 1B, links.lww.com/NXI/A712). We next tested AChR autoantibody–mediated MAC formation under the same conditions but with AChR-rapsyn expressed in HEK293T cells. An AChR-specific human recombinant IgG1 subclass mAb (mAb-637²⁸) and serum from an AChR autoantibody–positive patient (MG-87) were tested. In contrast to the results obtained in the AQP4 system, no AChR autoantibody–dependent MAC formation was detected (eFigure 1C, links.lww.com/NXI/A712).

Most human cells express the complement regulators CD46, CD55, and CD59, which can interfere with complement deposition. Therefore, we tested the complement CBA with CHO cells, which do not express these regulators.²⁹ An AChR-specific cell-based binding assay with CHO cells was established, but nonspecific background binding by human IgG prevented its use for complement detection (eFigure 2, A–D, links.lww.com/NXI/A712). A previous study demonstrated that the human HAP1 cell line, in which the expression of complement regulators was disrupted through gene editing, provided increased complement component deposition.³⁰ Thus, we reasoned that a genome-edited HEK293T cell line, in which the complement regulators CD46, CD55, and CD59 were not expressed, would be an effective tool in evaluating AChR autoantibody–mediated complement activity. Accordingly, we produced a modified HEK293T cell line using CRISPR/Cas9 genome editing to target these genes (eFigure 3, A–C, eTable 2, links.lww.com/NXI/A712). We then confirmed that autoantibody binding was uncompromised in the modified HEK293T cells (eFigure 4A, links.lww.com/NXI/A712) and that AChR was consistently expressed (eFigure 4B, links.lww.com/NXI/A712).

AChR Autoantibody–Mediated MAC Formation in the Absence of Complement Regulators

The CD46/55/59 KO HEK293T engineered cells were then tested in the complement CBA, first using the AQP4 antigen.

The AQP4-specific mAb-58 or serum from an AQP4 autoantibody-positive NMOSD patient was added to the cells and tested for MAC formation, as described for the WT HEK cells. MAC formation was detected when the AQP4-specific mAb or serum was added together with NHS. Conversely, MAC formation was not detected in the absence of either the AQP4 autoantibody source or NHS or when the mAb or serum was added with heat-inactivated NHS (Figure 1A). The AChR-specific mAb-637 and serum samples from a patient with MG and from healthy controls, which were tested under the same conditions, did not initiate MAC formation in the AQP4 complement CBA (Figure 1A).

To test the complement CBA with the modified cells for AChR autoantibody-mediated MAC formation, AChR and rapsyn were coexpressed, and the 637-mAb was added to the cells and tested for MAC formation. MAC formation was detected when the AChR-specific mAb and NHS were present (Figure 1B). We next tested MAC formation with serum samples from 4 AChR autoantibody-positive MG patients and 4 non-MG serum samples. Two were derived from MG patients (eTable 1, links.lww.com/NXI/A712) with AChR autoantibody titers—as measured by the clinical diagnostic radioimmunoassay—which were exceptionally high (MG-87 and MG-56, 43 and 88 nM, respectively) and 2 had mid- to low-range titers (MG-43 and MG-44, 14.3 and 1.47 nM, respectively). Serum from MG-87 mediated robust MAC formation (Figure 1C). Serum from MG-56 and MG-43 mediated weak MAC formation, and MG-44 showed none. HD serum, NMOSD serum, and the AQP4-specific mAb-58 did not mediate MAC formation on the AChR-expressing cells (Figure 1C).

To confirm that the antibody-mediated classical complement pathway was generating the MAC, we tested the contribution of the alternative pathway. This was achieved through inhibiting the alternative pathway by using NHS, in which factor B—required for the alternative pathway—was depleted. The MAC was detected when AQP4-specific mAb-58 and factor B-depleted serum were present but not in the absence of either the autoantibody or factor B-depleted serum or when the mAb was added with heat-inactivated factor B-depleted serum (Figure 1D). To confirm that the self-antigen was driving autoantibody binding and subsequent MAC formation, non-transfected cells were tested in the complement CBA using the AQP4-specific mAb-58, AChR-specific mAb-637, HD serum, and an MG patient serum sample (MG-87). MAC formation was below or at background levels using the nontransfected cells, indicating the requirement of antigen recognition in initiating antibody-mediated MAC formation (Figure 1E).

Testing MG Patient Serum for Autoantibody-Mediated MAC Formation

Having demonstrated that the complement CBA could measure AChR autoantibody-mediated classical pathway MAC formation, we next tested MG serum samples to explore how AChR autoantibody-mediated complement activity was represented in a heterogeneous population of MG patients. MAC

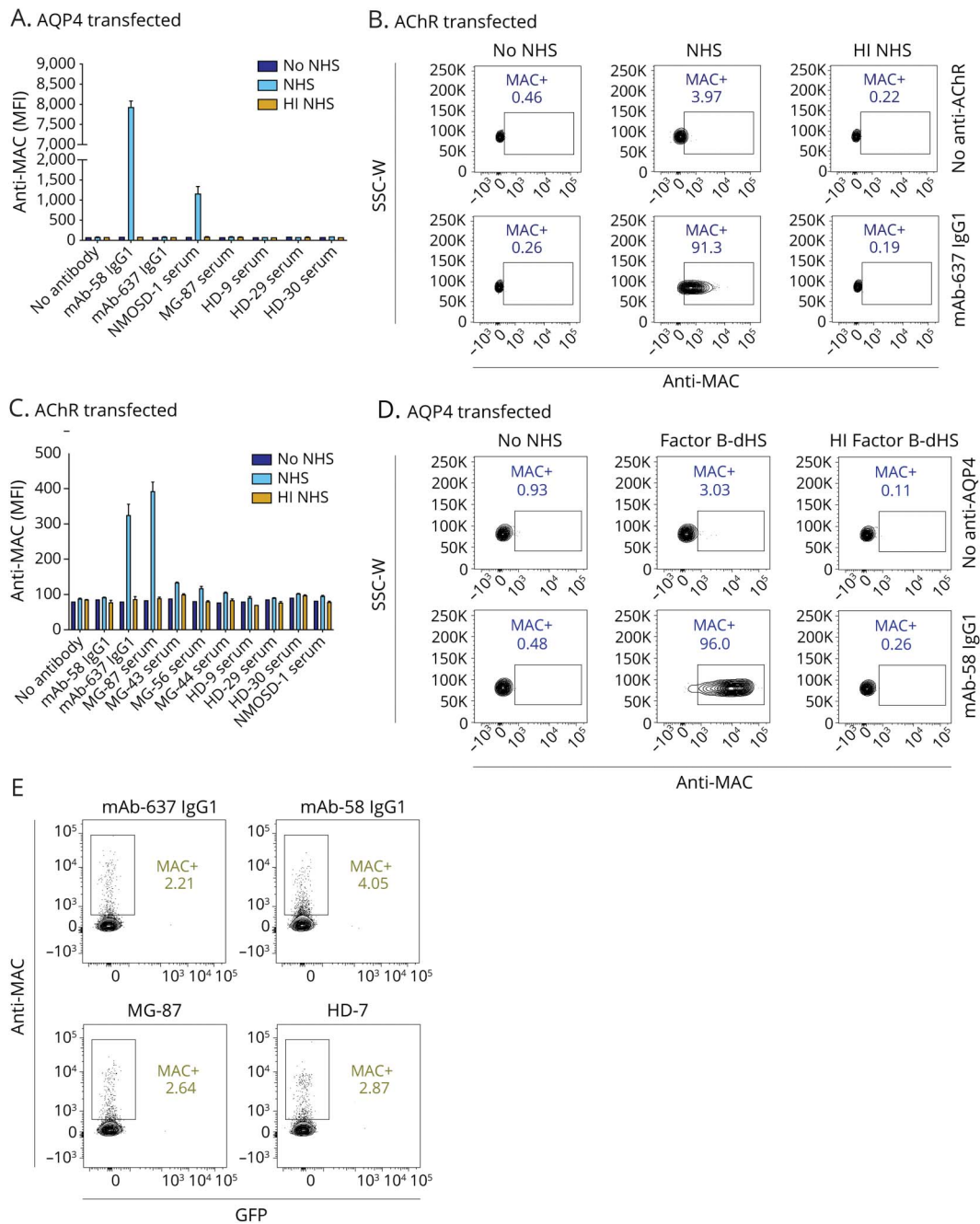
formation was tested using heat-inactivated serum from 30 HDs ($n = 32$ samples) and from 96 patients ($n = 155$ samples) with a confirmed diagnosis of AChR MG (eTable 1, links.lww.com/NXI/A712). While all MG patients were diagnosed with the AChR disease subtype, we specifically included patient samples that were AChR autoantibody-positive and others that were negative at the time of collection (eTable 1, links.lww.com/NXI/A712). An assay positivity cutoff was established using the MAC MFI from the HD group (156 ± 14 MFI); samples with an MFI $>$ mean + 4SD of the HD group were considered positive. Serum from the HD group did not show any measurable AChR autoantibody-mediated MAC formation (Figure 2A). A subset (83/155, 53.5%) of the MG patient-derived samples demonstrated AChR autoantibody-mediated MAC formation (Figure 2A). To determine whether those samples, which did not mediate MAC formation, included autoantibodies that were detectable by CBA, we next evaluated the association between MAC formation and AChR autoantibody binding by comparing the complement CBA results with those of the antibody-binding CBA. There was a significant correlation ($\rho = 0.897$; $p < 0.0001$) observed between MAC formation and AChR autoantibody binding (Figure 2B). Samples for which there was no detectable binding in the CBA did not mediate detectable MAC formation in the complement CBA (MAC formation was detected in 83/139 [59.7%] AChR-positive samples). In addition, samples that provided a positive, yet low, signal in the binding CBA did not mediate detectable MAC formation. Samples that were collected longitudinally presented a similar association pattern (eFigure 5, links.lww.com/NXI/A712).

Despite a strong correlation between MAC formation and AChR autoantibody binding (Figure 2B), we noted some samples with high MAC values despite relatively low AChR autoantibody binding and some with high AChR autoantibody binding with relatively low MAC values, which suggests a degree of heterogeneity among this population. To further explore the disassociation between MAC formation and AChR autoantibody binding, we examined the distribution of the ratio of MAC formation to AChR autoantibody binding. The ratios ranged between 0.27 and 48, with a median of 0.79 and an interquartile range of 0.43 (0.58–1.1), with an asymmetric, non-normal distribution with extreme values (skewness 5.1 and kurtosis 26 [leptokurtic]). The box-whisker plot (Figure 2C) shows relatively higher or lower ratios between MAC and AChR autoantibody binding beyond 10–90 percentiles, graphically representing the aforementioned heterogeneity among this population. These collective results suggest that, for most of the MG patient serum AChR autoantibodies, CBA binding positively associates with autoantibody-mediated CBA MAC formation, but a subset did not follow this pattern.

Comparing Titers of MG Patient Serum Autoantibody Binding and Autoantibody-Mediated MAC Formation

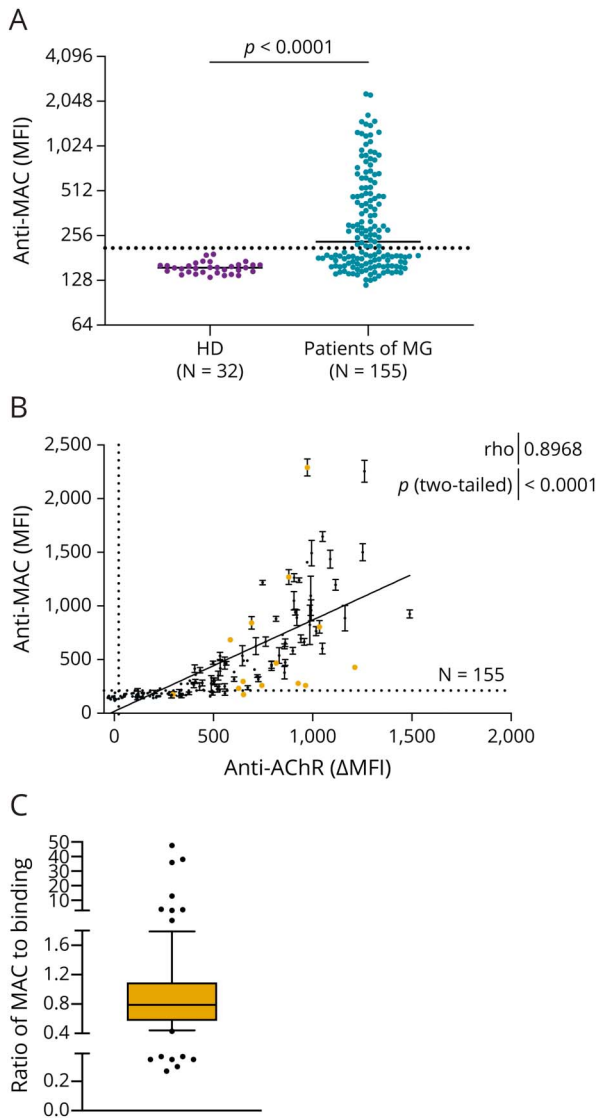
While CBA-based binding and MAC formation were closely associated for many samples, the results also suggest that a

Figure 1 Autoantibody-Mediated Complement Fixation Assay With CD46/55/59 KO HEK293T Cells



Autoantibodies were tested for their ability to mediate complement activation through measuring MAC formation through FACS on live cells, transfected with self-antigens, in which the complement regulators (CD46, CD55, and CD59) were knocked out. NHS was added as consistent complement source. Control conditions included omitted NHS or heat-inactivated NHS. All tested patient or HD serum samples were heat-inactivated. (A) Measurement of MAC formation using an in vitro complement CBA with AQP4 transfected CD46/55/59 KO HEK293T cells. Complement fixation was tested with AQP4-specific autoantibody sources (anti-AQP4 monoclonal mAb-58 IgG1 and NMOSD serum) and negative control autoantibody sources (anti-AChR mAb-637 IgG1 and serum from MG patients and HDs). NHS and HI NHS bars represent the mean of duplicate experimental conditions, whereas No NHS bars represent singlets. (B) Representative FACS analysis of AChR-transfected CD46/55/59 KO HEK293T cells using an in vitro complement CBA. AChR-transfected cells were tested without (top row) or with the anti-AChR mAb-637 IgG1 (bottom row). (C) Measurement of MAC formation using the complement CBA with AChR-transfected CD46/55/59 KO HEK293T. Complement fixation was tested with AChR-specific autoantibody sources (anti-AChR mAb-637 IgG1 and serum from MG patients and HDs). NHS and HI NHS bars represent the mean of duplicate experimental conditions, whereas No NHS bars represent singlets. (D) Factor B-depleted NHS was used in the complement CBA to test whether the alternative pathway contributed to autoantibody-mediated MAC formation. AQP4-transfected cells were tested without (top row) or with the anti-AQP4 monoclonal mAb-58 IgG1 (bottom row). (E) Nontransfected CD46/55/59 KO HEK293T cells were tested in the complement CBA using antigen-specific mAbs (mAb-637 or mAb-58) or serum from MG patients and HDs to test for the requirement of autoantigens in MAC formation. Samples used in panels (A-E) mAb-58 IgG1 (anti-AQP4 monoclonal), NMOSD patient (NMOSD-1), mAb-637 IgG1 (anti-AChR monoclonal), MG patient serum (MG-87, MG-43, MG-56, and MG-44), and healthy donors (HD-9, HD-29, and HD-30). AChR = acetylcholine receptor; blank = no serum; CBA = cell-based assay; factor B-dHS = factor B-depleted human serum, HDs = healthy donors; HI = heat-inactivated; HI factor B-dHS = heat-inactivated factor B-depleted human serum; MAC = membrane attack complex; MG = myasthenia gravis; NHS = normal human serum; NMOSD = neuromyelitis optica spectrum disorder.

Figure 2 AChR Autoantibody–Mediated Complement Formation in MG Patients



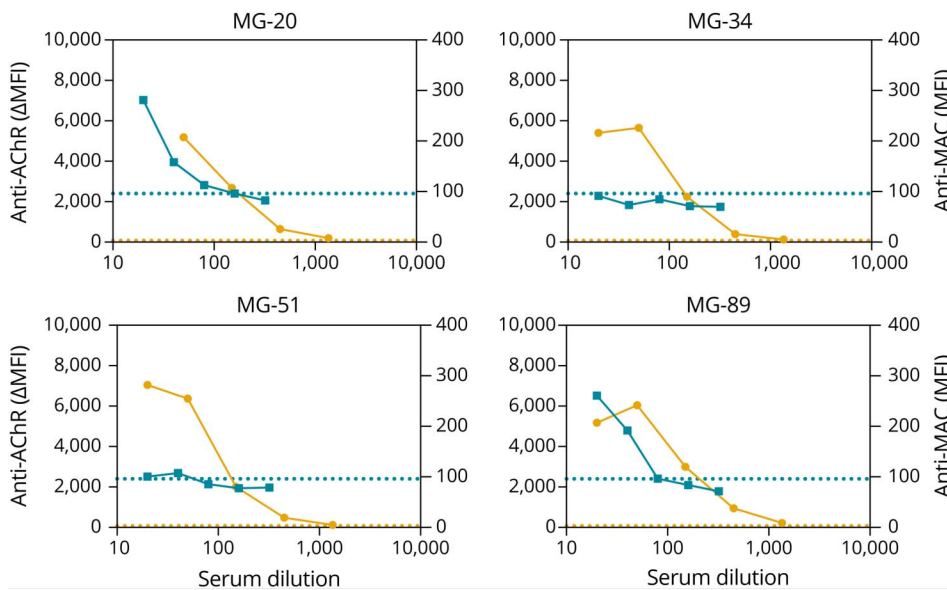
AChR autoantibodies in serum were tested for their ability to mediate complement activation through measuring MAC formation on live AChR-expressing CD46/55/59 KO HEK293T cells. (A) Comparison of MAC formation using serum samples ($n = 32$) from HDs and serum samples ($n = 155$) from AChR MG patients. The cutoff for positivity was set at the mean MFI +4 STD (210.9 MFI) of the HD samples. Each data point represents the mean of triplicate experimental conditions. (B) Correlation between MAC formation and AChR autoantibody binding by CBA. The Spearman correlation was used to calculate the relationship. The binding CBA cutoff for positivity was set at the mean MFI +4STD (21.91 Δ MFI) of the HD samples. Each data point represents the mean of triplicate experimental conditions. Colored (gold) points represent samples selected for further evaluation (Figure 3). (C) Graphical representation of heterogeneity in MAC formation and AChR autoantibody binding of MG samples. Cross-sectional samples ($n = 89$) were analyzed through a ratio of MAC formation and autoantibody binding to observe disassociation between AChR autoantibody binding and complement formation. The box-whisker plot shows the median (0.79) and 10–90 percentile (outlined by the whiskers) of the MAC to AChR autoantibody binding ratios. The dots represent data outside of the 10–90 percentile. The y-axis is not to scale and was divided into 3 sections for better visual representation. All samples were measured in triplicate, and those with negative Δ MFI binding values ($n = 5$) were excluded from the analysis. AChR = acetylcholine receptor; CBA = cell-based assay; HDs = healthy donors; MAC = membrane attack complex; MFI = mean fluorescence intensity; MG = myasthenia gravis.

subset of patients may harbor AChR binding autoantibodies that range broadly in their ability to mediate MAC formation. Accordingly, 14 samples were selected for further study (Figure 2B) based on their observed association/disassociation between the MAC formation and AChR autoantibody binding. Each sample was tested for AChR autoantibody binding and MAC formation at various serum dilutions (Figure 3, eFigure 6, links.lww.com/NXI/A712). The binding CBA, which tested serum over 3-fold dilutions from 1:20–1:1,350, showed a range of binding titers. The complement CBA, which tested serum over 2-fold dilutions from 1:20 to 1:320, showed a wide range of MAC formation. Of interest was that several samples showed similarly strong AChR autoantibody binding profiles but differed considerably in their MAC formation capability. For example, samples MG-20 and MG-34 have comparable binding profiles but considerably differ in their MAC formation capability; MG-20 mediated robust MAC formation at 3 of the serum dilutions tested, whereas MG-34 did not provide a signal above the cutoff at any serum dilution. Similarly, MG-49, MG-51, and MG-89 provided strong binding signals, but only MG-89 provided a strong MAC formation signal. These collective results imply that the autoantibodies harbored by these patients have differing pathogenic capacities irrespective of binding capability.

Association Between AChR Autoantibody Assay Data and Disease Severity, Treatment, or MG Subtypes

We first sought to explore the relationship between disease severity and autoantibody binding and autoantibody-mediated MAC formation. We first categorized the MG patients into 2 subgroups using the Myasthenia Gravis Foundation of American (MGFA) clinical classification: asymptomatic/ocular disease (MGFA = 0/I) and mild-to-severe generalized disease (MGFA \geq II) severity (eTable 1, links.lww.com/NXI/A712). Patients with higher disease severity showed both higher CBA binding and MAC formation when compared with those with minimal disease burden, as measured by the MGFA clinical classification ($p < 0.0001$ and $p < 0.0001$, respectively) (Figure 4, A and B). We next assessed the relationship between AChR autoantibody binding and MAC formation with the MG composite score (MGC)—a weighted disease severity score scale—by the Spearman correlation. Both binding and MAC formation showed mild-to-moderate correlation with MGC ($\rho = 0.437$, $p = 0.0001$; $\rho = 0.3512$, $p = 0.0023$, respectively) (Figure 4, C and 4D). Samples that were collected longitudinally presented a similar qualitative association between MAC formation, binding, and disease severity (eFigure 5, links.lww.com/NXI/A712). However, several samples in both the cross-sectional (Figure 4) and longitudinal (eFigure 5, links.lww.com/NXI/A712) analyses stood out because of their lack of a positive association, reflecting a similar disassociation to that which we observed between binding and MAC formation. For example, MG-37 had a moderate to high

Figure 3 Heterogeneous AChR Autoantibody–Mediated Complement Formation



Selected samples (indicated by golden points in Figure 2B) were examined side-by-side for both MAC formation and CBA binding. Each graph shows the data collected from individual serum samples. The x-axis represents the serum dilution of the sample tested for complement CBA (MAC) formation (left y-axis) or CBA binding (golden dots). For AChR autoantibody binding (left y-axis), samples were tested at serum dilutions of 1:20 and 4 additional 3-fold dilutions (1:50, 1:150, 1:450, and 1:1,350). For MAC formation (right y-axis), samples were tested at 2-fold serial dilutions (1:20, 1:40, 1:80, 1:160, and 1:320). Each data point represents the mean of experimental triplicate experimental conditions. Dotted horizontal lines mark the positive reactivity cutoff for complement (blue) and CBA binding (golden). Cutoffs were calculated using the mean MFI + 4STD of the HD samples (complement and CBA 96.01 MFI and 75.35 ΔMFI, respectively). Data from additional samples are shown in eFigure 6 (links.lww.com/NXI/A712). AChR = acetylcholine receptor; CBA = cell-based assay; HDs = healthy donors; MAC = membrane attack complex; MFI = mean fluorescence intensity.

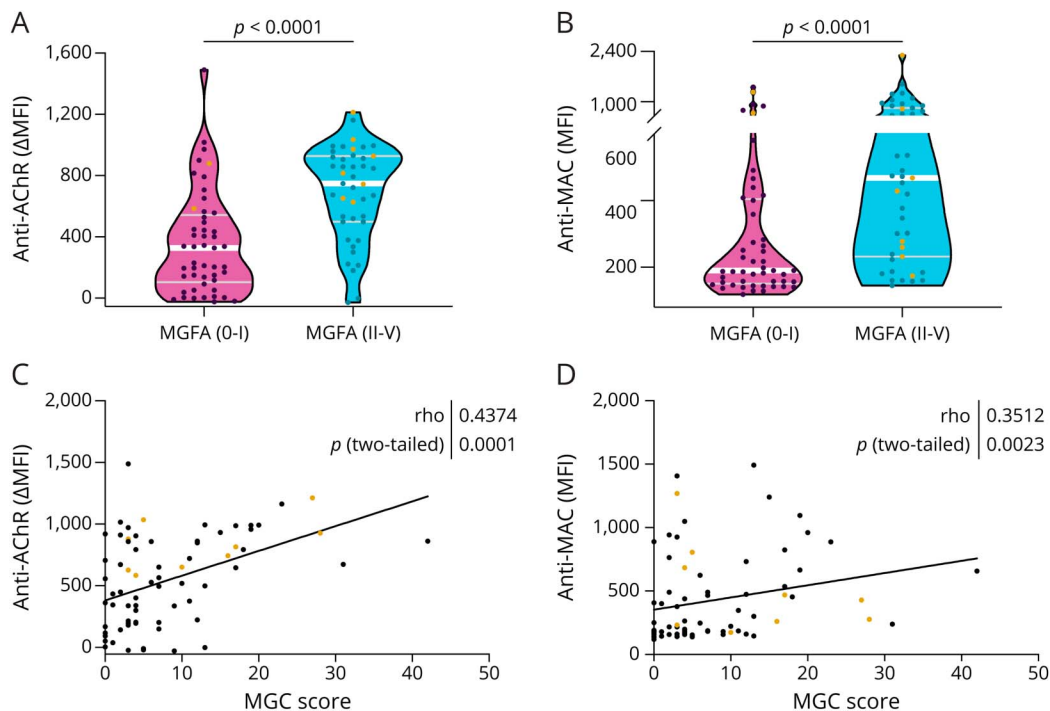
disease score, yet low antibody binding and MAC formation values. The opposite case was also found (MG-52) where both binding and MAC formation values were high relative to other samples, but the disease scores were low (eFigure 5, links.lww.com/NXI/A712). We also examined whether there were differences in autoantibody binding and autoantibody-complement-mediated MAC formation in specific MG patient subsets. Differences between treatment naïve and patients receiving immunotherapy, early-onset MG (EOMG) and late-onset MG (LOMG), and patients with or without thymectomy (eFigure 7, links.lww.com/NXI/A712) were evaluated. None of the subsets showed any significant differences regarding MAC formation and CBA binding. Collectively, these findings suggest that antibody-mediated complement activity moderately associates with disease burden, but some patients with mild and others with more severe disease have high or low autoantibody-mediated MAC formation activity, respectively.

Discussion

Detection of AChR autoantibodies confirms a diagnosis of MG; however, the discordance between titer and disease severity may be partly due to how AChR autoantibody titer is measured. Specifically, the clinical assays used to diagnose patients and provide titer values were unable to discriminate between the detection of the autoantibodies and their pathogenic properties (effector functions). Of importance, nonpathogenic AChR binding-only autoantibodies, which would be detected in clinical assays, may also exist, further complicating the disconnect between disease severity and autoantibody titer. Such

nonpathogenic binding autoantibodies have been identified in other autoimmune conditions including pemphigus³¹ and NMOSD.³² The nonpathogenic NMOSD autoantibodies, which are believed to be poor at activating complement due to altered Fc regions, compete with pathogenic autoantibodies for antigen binding.³² Our data revealed a positive association between autoantibody binding and MAC formation, suggesting that a considerable fraction of circulating AChR autoantibodies can initiate the complement cascade. However, binding alone did not account for the variation observed in complement activity. We reasoned that heterogeneous autoantibody characteristics, among other factors, may play an integral role in determining MAC formation efficiency. Such characteristics would minimally include subunit and epitope specificity, affinity, IgG subclass usage, Fc structure, and post-translation modifications, all of which can alter complement activating capability in addition to structure and stability.^{33,34} Although it is currently unclear how AChR autoantibody heterogeneity affects MAC formation in MG patients, parallels to the mechanisms governing AQP4 autoantibody-mediated pathology in NMOSD provide insight. AQP4-binding autoantibodies display broad efficiency in inducing complement-mediated cell death.²⁶ Here, a combination of distinct properties, including particular epitope binding and the assembly of multimeric antigen platforms, are required for ideal activation of autoantibody-complement-mediated cell death.²⁶ Specifically, AQP4 autoantibodies that target a distinct epitope on the AQP4 extracellular loop C demonstrate remarkably enhanced complement activity when compared with autoantibodies recognizing other epitopes. Furthermore, AQP4 can form supramolecular orthogonal arrays that arrange these epitopes in a manner that benefits

Figure 4 Correlation Between Clinical Disease Measurements and Autoantibody-Mediated Complement Formation



Cross-sectional samples were assessed for correlation between MGFA classification or MGC score and the binding CBA or complement CBA data. (A and B) Samples with low disease severity (MGFA 0/I) were compared with samples with higher disease severity (MGFA II–V) for differences in (A) autoantibody binding (median MGFA [0/I]: 327.3 [II–V]: 748.2; p -value < 0.0001) or (B) MAC formation (median MGFA [0/I]: 190.3 [II–V]: 468.3; p -value < 0.0001). (C and D) The Spearman correlation was used to calculate the relationship between cross-sectional sample MGC scores and (C) autoantibody binding (ρ 0.437, p -value = 0.0001) or (D) MAC formation (ρ 0.351, p -value = 0.0023). Each data point in (A–D) represents the mean of triplicate experimental conditions. White and gray lines in (A and B) represent the median and quartiles, respectively. CBA = cell-based assay; HDs = healthy donors; MAC = membrane attack complex; MGC = MG composite score; MGFA = Myasthenia Gravis Foundation of American.

autoantibody multimeric complexes, which support autoantibody Fc-Fc interactions that are critical for efficient C1q binding and subsequent complement activity.²⁶

Whether or not similar mechanisms occur in the context of AChR-specific MG has yet to be explored. However, given the variable complement activity shown here and that AChR is tightly clustered by the intracellular scaffolding protein, rapsyn, such organized formations of self-antigen may support Fc-Fc interactions facilitating efficient complement activation, with AChR autoantibodies recognizing particular—but not yet identified—epitopes. Thus, we proposed that circulating AChR autoantibodies include subtypes that contribute to heterogeneous pathogenetic mechanisms and show variable efficiency in affecting these mechanisms and, finally, that their composition varies among patients and perhaps longitudinally within a patient. We further suggested that AChR autoantibody subunit specificity and epitope specificity are linked to these effector functions, including activation of the classical complement pathway.

We recognized that this study included limitations. First, measuring complement formation on live cells with flow cytometry presented challenges, given that the cells may be actively lysing or dying and therefore not detected. We empirically arrived at a single time point

after the addition of NHS that provided measurable differences in MAC formation in the AQP4 and AChR complement CBAs when comparing patient samples and controls. This approach was chosen in consideration of assay ease of use.

Longitudinal measurements after the addition of NHS or different approaches that measure cumulative cell death, such as Cr⁵² release, may provide additional information on AChR autoantibody-mediated activity, but were outside the scope of this study. Second, autoantibody-mediated MAC formation was not detected in a number of samples, most of which showed lower, but still positive, CBA binding. It is reasonable to conclude that these samples do indeed include AChR autoantibodies capable of mediating MAC formation. However, the sensitivity of the complement CBA may require further optimization to detect their activity. Finally, we recognize that the in vitro autoantibody-mediated complement activity may not fully reflect an in vivo complement response. We are using an in vitro system with diminished expression of complement inhibitory proteins. Our system reflects the capability of the autoantibody to initiate complement but does not consider the varying levels of complement inhibitory protein expression on various muscle cell surfaces. In this regard, the assay only simulates more complex activity at the NMJ; however, the clinical binding CBA also lacks any representation of the

NMJ other than antigen expression, but it is recognized as being highly reliable for diagnosis of MG. Further emphasizing this point, it is challenging to understand why it was required to remove the complement regulators because they are present in the NMJ. It may be related to *in vitro* assay sensitivity, but further study is required to understand this observation. Of interest, this requirement has been consistently observed in other studies that investigated pathophysiologic properties of AChR autoantibodies³⁵ using *in vitro* assays and *in vivo* models; specifically, CD55 knockout mice, which are more susceptible to the effects of pathogenic MG autoantibodies.³⁶⁻³⁸ These regulators have also been implicated in ocular MG, where reduced expression levels of CD55 and CD59 in extraocular muscles offer a potential explanation for why these muscle subgroups are highly associated with MG.³⁶ To reflect the NMJ-specific conditions more accurately, our assay could be complemented by other approaches. For example, motor neurons—the target cells in patients with multifocal motor neuropathy (MMN)—have been used for investigating the pathogenicity of MMN autoantibodies.³⁹ In a similar fashion, functional consequences of complement damage could be assessed with NMJ-specific cell types.

We observed a modest correlation between autoantibody-mediated complement activity and disease burden. While additional mechanisms must be considered, this suggests that in some patients, complement activity may make major contributions to pathology. Conversely, patients who did not fit this pattern, for example, those with high disease burden and low complement activity may harbor autoantibodies that mediate pathology through other mechanisms (blocking and/or modulating).

Furthermore, we did not observe differences in autoantibody-mediated complement activity between EOMG and LOMG, which suggests that the properties of the autoantibodies may be similar in these major MG subsets. Immunomodulatory therapy and thymectomy did not reveal diminished complement activity. Autoantibody titer often persists after such treatments^{40,41}; thus, our findings suggest that these treatments may not alter either the properties or relative frequency of complement activating autoantibodies.

It is anticipated that measuring AChR autoantibody-mediated pathogenesis will have clinical utility. The past decade has seen a considerable number of new and existing therapeutics become available for the treatment of MG.¹⁶ Included are biologics that directly target the autoantibody effector function (eculizumab and others).⁴² Clinical trials of these treatments have provided outcomes demonstrating benefits in some patients but unexpected failures in others, thus intensifying challenges to the field, including the need to target the correct therapy to patients from the growing number of options and the need to inform treatment decisions through understanding the immunopathology. Therapeutic complement inhibition provides one such example of our limited understanding of AChR MG immunomechanisms: it is unclear why eculizumab (approved for the

treatment of generalized AChR MG¹⁷) does not yield clinical benefit in some AChR autoantibody-positive patients,^{17,18} even with longer-term treatment.¹⁹ The AChR autoantibody titers of patients who responded to eculizumab treatment neither predicted treatment efficacy nor correlated with its progression. In addition to emphasizing the limitations of AChR autoantibody titer as a biomarker, these studies also underscore the need to further understand AChR autoantibody mechanisms so that response to treatments can be better predicted. Future experiments, which include testing samples from complement inhibitor trials, could be performed to investigate whether the complement CBA is valuable in forecasting response. To this end, we suggest a suite of clinical assays that measure the composition of AChR autoantibodies with variable pathogenic mechanisms will be valuable. These assays would ideally measure binding-only, classical complement activation as shown here, and modulating and blocking functions, which have been demonstrated with similar CBA platforms.^{43,44} These collective measurements may be useful for assessing disease progression and serve as an improved MG biomarker when compared with autoantibody binding. Thus, patients would be provided with the opportunity for a more individualized treatment plan that targets their unique autoantibody-mediated pathogenic pathways.

Acknowledgment

The authors thank Drs. Soumya Yandamuri, Gianvito Masi, Philip Coish, and Ms. Sarah Ohashi for critical reading of the article.

Study Funding

KCO was supported by the National Institute of Allergy and Infectious Diseases of the NIH under award numbers R01-AI114780 and R21 AI164590; through an MGNet pilot grant awarded through the Rare Diseases Clinical Research Consortium of the NIH (award number US4-NS115054) and by a High Impact Clinical Research and Scientific Pilot Project award from the Myasthenia Gravis Foundation of America (MGFA). This project was also supported partly by grants to KCO and RJN from Ra Pharmaceuticals, now a part of UCB. JLB was supported by the National Eye Institute R01-EY022936 and R21-032399. MLF was supported partly by the SPIN award through Grifols and has further been supported by a DFG Research fellowship (FI 2471/1-1).

Disclosure

Abeer H. Obaid reports no disclosures; Dr. Chryssa Zografou reports no disclosures; Dr. Douangson D. Vadysirisack is an employee of UCB Ra Pharma; Dr. Bailey Munro-Sheldon reports no disclosures; Dr. Miriam L. Fichtner reports no disclosures; Dr. Roy is a consultant for Alexion Pharmaceuticals (now a part of AstraZeneca) and Takeda Pharmaceuticals; Dr. William M. Philbrick reports no disclosures; Dr. Jeffrey L. Bennett reports payment consultation fees for study design/consultation from Viela Bio, now a part of Horizon Therapeutics, and personal fees from Mitsubishi Tanabe Pharma Corporation, Reistone Bio, AbbVie, Alexion, Chugai, Clene Nanomedicine, Genentech, Genzyme, Mitsubishi-

Tanabe, and Roche, grants from Novartis, Mallinckrodt, and has a patent for Aquaporin issued; Dr. Richard J. Nowak has received research support from the NIH, Genentech, Alexion Pharmaceuticals, argenx, Annexon Biosciences, UCB Ra Pharmaceuticals, Myasthenia Gravis Foundation of America, Momenta, Immunovant, Grifols, and Viela Bio, now a part of Horizon Therapeutics. RJN has served as consultant/advisor for Alexion Pharmaceuticals, argenx, Cabaletta Bio, CSL Behring, Grifols, Ra Pharmaceuticals, now a part of UCB Pharma, Immunovant, Momenta, and Viela Bio, now a part of Horizon Therapeutics; Dr. Kevin C. O'Connor has received research support from Ra Pharma, now a part of UCB Pharma, and Alexion, now a part of AstraZeneca, and Viela Bio, now a part of Horizon Therapeutics. KCO is a consultant and equity shareholder of Cabaletta Bio. KCO has served as a consultant/advisor for Alexion Pharmaceuticals, now a part of AstraZeneca, and Roche, and he has received speaking fees from Alexion, Roche, Genentech, and Viela Bio, now a part of Horizon Therapeutics. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* October 11, 2021. Accepted in final form March 8, 2022. Submitted and externally peer reviewed. The handling editor was Marinos C. Dalakas, MD, FAAN.

Appendix Authors

Name	Location	Contribution
Abeer H. Obaid, BS	Department of Neurology, Yale School of Medicine, New Haven, CT; Department of Immunobiology, Yale School of Medicine, New Haven, CT; Institute of Biomedical Studies, Baylor University, Waco, TX	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Chryssa Zografou, PhD	Department of Neurology, Yale School of Medicine, New Haven, CT; Department of Immunobiology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data; and additional contributions: CZ performed the CRISPR experiments
Douangsone D. Vadysirisack, PhD	UCB Pharma, Cambridge, MA	Drafting/revision of the article for content, including medical writing for content; study concept or design; additional contributions: DV contributed to experimental direction and design
Bailey Munro-Sheldon, PhD, MS	Department of Neurology, Yale School of Medicine, New Haven, CT	Analysis or interpretation of data; additional contributions: BMS contributed to the clinical data acquisition

Appendix (continued)

Name	Location	Contribution
Miriam L. Fichtner, MD	Department of Neurology, Yale School of Medicine, New Haven, CT; Department of Immunobiology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data; additional contributions: MF conducted the CHO cells-related experiments
Bhaskar Roy, MBBS, MHS	Department of Neurology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; study concept or design; analysis or interpretation of data; and additional contributions: conducted statistical analysis
William M. Philbrick, PhD	Department of Immunobiology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; study concept or design; additional contributions: WP contributed to the CRISPR gene editing design
Jeffrey L. Bennett, MD, PhD	Departments of Neurology and Ophthalmology, Programs in Neuroscience and Immunology, University of Colorado Anschutz Medical Campus, Aurora, CO	Drafting/revision of the article for content, including medical writing for content; additional contributions: JB provided NMOSD monoclonal and guidance on the article
Richard J. Nowak, MD, MS	Department of Neurology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data; and additional contributions: RN contributed in acquisition of clinical specimens and associated clinical data and project direction
Kevin C. O'Connor, PhD	Department of Neurology, Yale School of Medicine, New Haven, CT; Department of Immunobiology, Yale School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

References

- Vincent A. Unravelling the pathogenesis of myasthenia gravis. *Nat Rev Immunol*. 2002; 2(10):797-804.
- Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis—autoantibody characteristics and their implications for therapy. *Nat Rev Neurol*. 2016;12(5):259-268.
- Yi JS, Guptill JT, Stathopoulos P, Nowak RJ, O'Connor KC. B cells in the pathophysiology of myasthenia gravis. *Muscle Nerve*. 2018;57(2):172-184.
- Vincent A, Newsom Davis J. Anti-acetylcholine receptor antibodies. *J Neurol Neurosurg Psychiatry*. 1980;43(7):590-600.
- Lefvert AK, Bergström K, Matell G, Osterman PO, Pirskanen R. Determination of acetylcholine receptor antibody in myasthenia gravis: clinical usefulness and pathogenetic implications. *J Neurol Neurosurg Psychiatry*. 1978; 41(5):394-403.
- Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology*. 1976;26(11):1054-1059.
- Tindall RS. Humoral immunity in myasthenia gravis: clinical correlations of anti-receptor antibody avidity and titer. *Ann N Y Acad Sci*. 1981;377:316-331.

8. Tzartos SJ, Barkas T, Cung MT, et al. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunol Rev*. 1998;163:89-120.
9. Lindstrom JM, Engel AG, Seybold ME, Lennon VA, Lambert EH. Pathological mechanisms in experimental autoimmune myasthenia gravis. II. Passive transfer of experimental autoimmune myasthenia gravis in rats with anti-acetylcholine receptor antibodies. *J Exp Med*. 1976;144(3):739-753.
10. Sterz R, Hohlfeld R, Rajki K, et al. Effector mechanisms in myasthenia gravis: end-plate function after passive transfer of IgG, Fab, and F(ab')₂ hybrid molecules. *Muscle Nerve*. 1986;9(4):306-312.
11. Howard FM Jr, Lennon VA, Finley J, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis. *Ann N Y Acad Sci*. 1987;505:526-538.
12. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N Engl J Med*. 1978;298(20):1116-1122.
13. Drachman DB, Adams RN, Josifek LF, Self SG. Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N Engl J Med*. 1982;307(13):769-775.
14. Kao I, Drachman DB. Myasthenic immunoglobulin accelerates acetylcholine receptor degradation. *Science*. 1977;196(4289):527-529.
15. Gomez CM, Richman DP. Anti-acetylcholine receptor antibodies directed against the alpha-bungarotoxin binding site induce a unique form of experimental myasthenia. *Proc Natl Acad Sci U S A*. 1983;80(13):4089-4093.
16. Nguyen-Cao TM, Gelin D, Griffin R, Mondou E. Myasthenia gravis: historical achievements and the "golden age" of clinical trials. *J Neurol Sci*. 2019;406:116428.
17. Howard JF Jr, Utsugisawa K, Benatar M, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol*. 2017;16(12):976-986.
18. Muppidi S, Utsugisawa K, Benatar M, et al. Long-term safety and efficacy of eculizumab in generalized myasthenia gravis. *Muscle Nerve*. 2019;60(1):14-24.
19. Howard JF Jr, Karam C, Yountz M, O'Brien FL, Mozaffar T, Group RS. Long-term efficacy of eculizumab in refractory generalized myasthenia gravis: responder analyses. *Ann Clin Transl Neurol*. 2021;8(7):1398-1407.
20. Anil R, Kumar A, Alaparthy S, et al. Exploring outcomes and characteristics of myasthenia gravis: rationale, aims and design of registry—the EXPLORE-MG registry. *J Neurol Sci*. 2020;414:116830.
21. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013;8(11):2281-2308.
22. Stathopoulos P, Chastre A, Waters P, et al. Autoantibodies against neurologic antigens in nonneurologic autoimmunity. *J Immunol*. 2019;202(8):2210-2219.
23. Takata K, Stathopoulos P, Cao M, et al. Characterization of pathogenic monoclonal autoantibodies derived from muscle-specific kinase myasthenia gravis patients. *JCI Insight*. 2019;4(12):e127167.
24. von Elm EAD, Egger M, Pocock SJ, Gotsche PC, Vandembroucke JP. The strengthening of reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ*. 2007;335:806.
25. Rodriguez Cruz PM, Huda S, Lopez-Ruiz P, Vincent A. Use of cell-based assays in myasthenia gravis and other antibody-mediated diseases. *Exp Neurol*. 2015;270:66-71.
26. Soltys J, Liu Y, Ritchie A, et al. Membrane assembly of aquaporin-4 autoantibodies regulates classical complement activation in neuromyelitis optica. *J Clin Invest*. 2019;129:2000-2013.
27. Bennett JL, Lam C, Kalluri SR, et al. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol*. 2009;66(5):617-629.
28. Graus YF, de Baets MH, Parren PW, et al. Human anti-nicotinic acetylcholine receptor recombinant Fab fragments isolated from thymus-derived phage display libraries from myasthenia gravis patients reflect predominant specificities in serum and block the action of pathogenic serum antibodies. *J Immunol*. 1997;158(4):1919-1929.
29. Saifuddin M, Hedayati T, Atkinson JP, Holguin MH, Parker CJ, Spear GT. Human immunodeficiency virus type 1 incorporates both glycosyl phosphatidylinositol-anchored CD55 and CD59 and integral membrane CD46 at levels that protect from complement-mediated destruction. *J Gen Virol*. 1997;78(pt 8):1907-1911.
30. Thielen AJF, van Baarsen IM, Jongasma ML, Zeerleder S, Spaapen RM, Wouters D. CRISPR/Cas9 generated human CD46, CD55 and CD59 knockout cell lines as a tool for complement research. *J Immunol Methods*. 2018;456:15-22.
31. Payne AS, Ishii K, Kacir S, et al. Genetic and functional characterization of human pemphigus vulgaris monoclonal autoantibodies isolated by phage display. *J Clin Invest*. 2005;115(4):888-899.
32. Tradtrantip L, Yeaman MR, Verkman AS. Cytoprotective IgG antibodies in sera from a subset of patients with AQP4-IgG seropositive neuromyelitis optica spectrum disorder. *Sci Rep*. 2021;11(1):21962.
33. Alter G, Ottenhoff THM, Joosten SA. Antibody glycosylation in inflammation, disease and vaccination. *Semin Immunol*. 2018;39:102-110.
34. Quast I, Keller CW, Maurer MA, et al. Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity. *J Clin Invest*. 2015;125(11):4160-4170.
35. Jacob S, Viegas S, Leite MI, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. *Arch Neurol*. 2012;69(8):994-1001.
36. Kaminski HJ, Li Z, Richmonds C, Lin F, Medof ME. Complement regulators in extraocular muscle and experimental autoimmune myasthenia gravis. *Exp Neurol*. 2004;189(2):333-342.
37. Lin F, Kaminski HJ, Conti-Fine BM, Wang W, Richmonds C, Medof ME. Markedly enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. *J Clin Invest*. 2002;110(9):1269-1274.
38. Kaminski HJ, Kusner LL, Richmonds C, Medof ME, Lin F. Deficiency of decay accelerating factor and CD59 leads to crisis in experimental myasthenia. *Exp Neurol*. 2006;202(2):287-293.
39. Harschnitz O, van den Berg LH, Johansen LE, et al. Autoantibody pathogenicity in a multifocal motor neuropathy induced pluripotent stem cell-derived model. *Ann Neurol*. 2016;80(1):71-88.
40. Nowak RJ, Coffey CS, Goldstein JM, et al. Phase 2 trial of rituximab in acetylcholine receptor antibody-positive generalized myasthenia gravis: the BeatMG study. *Neurology*. 2021;98(4):e376-e389.
41. Jiang R, Hoehn KB, Lee CS, et al. Thymus-derived B cell clones persist in the circulation after thymectomy in myasthenia gravis. *Proc Natl Acad Sci U S A*. 2020;117(48):30649-30660.
42. Howard JF Jr, Barohn RJ, Cutter GR, et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. *Muscle Nerve*. 2013;48:76-84.
43. Lozier BK, Haven TR, Astill ME, Hill HR. Detection of acetylcholine receptor modulating antibodies by flow cytometry. *Am J Clin Pathol*. 2015;143(2):186-305. quiz 305.
44. Cromar A, Lozier BK, Haven TR, Hill HR. Detection of acetylcholine receptor blocking antibodies by flow cytometry. *Am J Clin Pathol*. 2016;145(1):81-85.

Neurology[®] Neuroimmunology & Neuroinflammation

Heterogeneity of Acetylcholine Receptor Autoantibody–Mediated Complement Activity in Patients With Myasthenia Gravis

Abeer H. Obaid, Chryssa Zografou, Douangsone D. Vadysirisack, et al.
Neurol Neuroimmunol Neuroinflamm 2022;9;
DOI 10.1212/NXI.0000000000001169

This information is current as of April 26, 2022

Updated Information & Services	including high resolution figures, can be found at: http://nn.neurology.org/content/9/4/e1169.full.html
References	This article cites 44 articles, 9 of which you can access for free at: http://nn.neurology.org/content/9/4/e1169.full.html##ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Autoimmune diseases http://nn.neurology.org/cgi/collection/autoimmune_diseases Myasthenia http://nn.neurology.org/cgi/collection/myasthenia
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://nn.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://nn.neurology.org/misc/addir.xhtml#reprintsus

Neurol Neuroimmunol Neuroinflamm is an official journal of the American Academy of Neurology. Published since April 2014, it is an open-access, online-only, continuous publication journal. Copyright Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2332-7812.

