RESEARCH ARTICLE OPEN ACCESS

N-Glycan–Dependent Proinflammatory Effects of IgM in Anti-MAG Neuropathy

Jean Neil,[1,2,1]* François Fenaille,[3] Arnaud Bruneel,[4,5] Tanya Stojkovic,[6] Sophie Cholet,[3] Emilien Delmont,[7] Pauline Ober,[1] Alexandre Raynor,[4] Quentin Amiot,[1] Karim Dorgham,[2] Karine Viala,[6,8] Pascale Ghillani-Dalbin,[1] Guy Gorochov,[1,2] and Delphine Sterlin[1,2,2]*

Correspondence
Dr. Neil
jean.neil@aphp.fr

Neurol Neuroimmunol Neuroinflamm 2025;12:e200440. doi:10.1212/NXI.0000000000200440

Abstract

Background and Objectives

Anti–myelin-associated glycoprotein (anti-MAG) neuropathy is a chronic demyelinating neuropathy with deposits of IgM and sural nerve fiber demyelination. While growing evidence supports the critical role of IgG glycosylation in autoimmune diseases, IgM glycosylation profiles markedly differ from those of IgG but are largely neglected. The aim of this study was to characterize IgM N-glycosylation in patients with anti-MAG neuropathy and its involvement in anti-MAG pathogenicity.

Methods

IgM antibodies were isolated from patients with anti-MAG neuropathy (n = 17), asymptomatic patients with monoclonal gammopathy of undetermined significance (n = 8), and healthy donors ([HDs], n = 6). N-glycan analysis was performed using mass spectrometry. Binding to myelin-associated glycoprotein (MAG), complement (C1q), and IgM Fc receptors (Fcα/μR and DC-SIGN) was compared using ELISA between anti-MAG and MGUS IgM, before and after N-deglycosylation/desialylation. Finally, we assessed how IgM N-glycosylation influences cytokine production by monocyte-derived macrophages by measuring cytokine levels in culture supernatants.

Results

Anti-MAG IgM exhibited a unique glycosylation pattern, dominated by fucosylated, monosialylated N-glycan with a bisecting N-acetyl glucosamine (N-glycan 12), representing 48.5% of the total N-glycan pool, compared with 27.3% in MGUS IgM and 35.6% in HD IgM. We showed that deglycosylation and desialylation significantly reduced anti-MAG activity and C1q binding (average % of decrease 58.3 ± 18.8, $p < 0.01$, and 40.0 ± 19.9%, $p < 0.05$, respectively). Furthermore, anti-MAG IgM binding to C1q was significantly higher than that of MGUS IgM and HD IgM ($p < 0.0001$ and $p < 0.001$, respectively). Compared with MGUS IgM, anti-MAG IgM also significantly increased the production of proinflammatory cytokines IL-1, IL-6, IL-8, TNF-α, and IFN-ϒ by macrophages, in a glycan-dependent manner ($p < 0.01$ to $p < 0.001$), with IL-8 being particularly elevated. Finally, we found that anti-MAG IgM bound more strongly Fcα/μ receptor and DC-SIGN compared with MGUS IgM ($p < 0.05$ and $p = 0.06$).

Discussion

This study uncovered a unique N-glycosylation pattern of anti-MAG IgM, crucial for its interaction with MAG and binding to C1q. Moreover, anti-MAG IgM increased the macrophage cytokine production, driven by their glycosylation. The increased IL-8 expression and

MORE ONLINE

Supplementary Material

*These authors contributed equally to this work.

[1]Département d'Immunologie, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital La Pitié-Salpêtrière, Sorbonne Université, France; [2]Inserm, CNRS, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Sorbonne Université, France; [3]Département Médicaments et Technologies pour La Santé (DMTS), Université Paris-Saclay, CEA, INRAE, MetaboHUB, France; [4]AP-HP, Biochimie Métabolique et Cellulaire, Hôpital Bichat, Paris, France; [5]Université Paris-Saclay, Inserm UMR1193, Faculté de Pharmacie, Orsay, France; [6]AP-HP, Centre de référence des maladies neuromusculaires NORD/EST/Ile de France, Hôpital Pitié-Salpêtrière, Sorbonne Université, Paris, France; [7]Referral centre for neuromuscular diseases and ALS, La Timone hospital, Aix-Marseille University, ERN-NMD, FILNEMUS, France; and [8]AP-HP, département de neurophysiologie, département de neurologie, Hôpital Pitié-Salpêtrière, Sorbonne Université, Paris, France.

The Article Processing Charge was funded by Assistance Publique-Hôpitaux de Paris.

Glossary

BKT = bruton tyrosine kinase; **BTU** = Bühlmann titer unit; **HD** = healthy donor; **MAG** = myelin-associated glycoprotein; **MALDI-TOF MS** = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; **MGUS** = monoclonal gammopathy of undetermined significance; **OD** = optical density; **ONLS** = Overall Neuropathy Limitation Scale; **PBS** = phosphate buffer saline; **pIgR** = polymeric immunoglobulin receptor; **RODS** = Rasch-Built Overall Disability Scale; **SGPG** = sulphated glucuronyl paragloboside; **SIGLEC** = sialic acid–binding immunoglobulin-like lectin.

anti-MAG IgM binding to C1q might open 2 potential therapeutic avenues: inhibiting IL-8 activity or targeting the complement pathway. In addition, the glycosylation and C1q binding of anti-MAG IgM could serve as biomarkers for monitoring this neuropathy.

Introduction

Immunoglobulin glycosylation is one of the most common post-translational modifications involved in numerous diseases, especially certain autoimmune diseases.[1] Variations in mannosylation, galactosylation, fucosylation, and sialylation drive differential Ig functions, ranging from complement activation, cytokine production, and anti-inflammatory or proinflammatory effects.[2,3] IgG serves as a key example of how changes in immunoglobulin glycosylation influence downstream immune responses. Although the glycosylation of IgG has been extensively studied in autoimmunity, the glycosylation profile of IgM remains unclear owing to the complexity of this pentameric protein. Human pentameric IgM contains 5 N-glycosylation sites located on the crystallizable fragment (Fc) of the μ (heavy) chain (Asn-171, Asn-332, Asn-395, Asn-402, and Asn-563) and 1 N-glycosylation site on the J chain. In healthy human serum, complex-type N-glycans are attached to N-glycosylation sites except Asn-402 and Asn-563, which bear mannosylated N-glycans.[4,5] N-glycosylation of IgM has been further characterized during cancer,[6,7] acute SARS-CoV-2 infection, and Lyme disease.[8,9] A study performed in 2015[10] showed that increased IgM sialylation abolishes T-cell activation. More recently, IgM N-glycans were linked to antibody-dependent complement deposition rate during acute viral infection.[9] However, the extent to which IgM N-glycans influence IgM effector functions and interactions with its receptors remains enigmatic.

Anti–myelin-associated glycoprotein (anti-MAG) IgM monoclonal antibodies have been associated with a slowly progressive sensory ataxic neuropathy, so-called anti-MAG neuropathy. Sensory distal demyelinating neuropathy with ataxia and sometimes postural tremor predominate this well-recognized clinical entity and can lead to serious disability.[11,12] Immunologic treatments, including polyvalent immunoglobulins, rituximab, fludarabine, and plasma exchange, may improve nerve conduction and control symptoms while new therapies are evaluated in ongoing clinical trials.[13]

MAG is a transmembrane glycoprotein expressed on the periaxonal surface of oligodendrocytes and Schwann cells.[14] The extracellular part of MAG contains 8 carbohydrate moieties including the human natural killer-1 (HNK-1), which is the target of anti-MAG IgM autoantibodies.[15,16] In addition to IgM immunoreactivity for the HNK-1 epitope, MAG shows affinity for N-linked oligosaccharides with 2,3-sialic acid and 2,6-sialic acid (2,3-SA and 2,6-SA, respectively) found on glycoproteins and some gangliosides, as a member of the sialic acid–binding immunoglobulin-like lectin (SIGLEC4) family.[14] It also binds to an acidic glycolipid in peripheral nerves, identified as sulphated glucuronyl paragloboside (SGPG),[17,18] which shares the HNK-1 epitope. Although IgM paraproteins recognize both MAG and SGPG,[19,20] this study primarily focuses on MAG because IgM binds to MAG 10 to 100 times more strongly than to SGPG.[19] The pathogenicity of anti-MAG antibodies has been demonstrated in several studies, including passive and active animal model immunizations[21,22] that highlight a link between anti-MAG IgM and complement activation. However, the molecular mechanisms underlying anti-MAG pathogenicity are still poorly understood.

In this study, we hypothesized that IgM N-glycans may play a role in the pathogenicity of anti-MAG antibodies. We highlighted N-glycan structures associated with anti-MAG IgM and compared them with those carried by IgM from patients with monoclonal gammopathy of undetermined significance (MGUS) and healthy donors (HDs). We then evaluated the contribution of N-glycans to the binding of anti-MAG IgM to MAG, C1q, and IgM Fc receptors. Finally, we investigated how anti-MAG IgM modulates cytokine production by macrophages.

Methods

Patients

In this study, 17 patients with anti-MAG neuropathy, 8 with IgM MGUS without any clinical involvement, and 6 healthy donors (HDs) have been enrolled. Patients with anti-MAG neuropathy and MGUS were age/sex-matched, but HDs were younger. They were recruited at either the Department of

Neurology of Pitié-Salpêtrière Hospital (Paris, France) or the Department of Neurology of La Timone Hospital (Marseille, France). Anti-MAG patients fulfilled the electromyographic criteria from the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) guidelines for chronic inflammatory demyelinating polyradiculoneuropathy.[23] Some patients were diagnosed following the 2010 EFNS/PNS criteria, but these diagnoses were not contradicted by the 2021 criteria.[24] Patient disability was assessed using the Overall Neuropathy Limitation Scale (ONLS) and the Rasch-Built Overall Disability Scale (RODS). Serum samples were collected at a single time point and stored at −20°C until use. Detailed patient information is summarized in the Table.

IgM Extraction

IgM was extracted from serum samples as previously described.[25] In brief, serum samples were passed through anti-human IgM (μ-chain–specific) agarose columns (Sigma-Aldrich, St Louis, US). Affinity chromatography steps were performed at a flow rate of 0.5 mL/minute according to the manufacturer's instructions. IgM was then eluted with 0.1M glycine pH 2.5 (Sigma-Aldrich), and neutral pH was immediately adjusted with 1M Tris pH 8. Buffer exchange was achieved using dialysis membranes in 1X-phosphate buffer saline (PBS), pH 7.4 at 4°C, 2 times for 6 hours each. IgM quantification was performed by both spectrophotometry absorbance at 280 nm ($E^{1\%}$ -IgM = 1.18) and nephelometric assay (Siemens BN II, IgM, IgG, and IgA assays). These 2 methods confirmed IgM purity; i.e., undesirable isotypes (IgG and IgA) represented less than 1% of the purified immunoglobulins. Finally, electrophoresis in native conditions was performed to verify IgM pentameric format. Purified IgM was separated using 4%–20% polyacrylamide gel (Mini-PROTEAN TGX Stain-Free Precast Gels, Bio-Rad) and revealed with Imperial Protein Stain (Thermo Scientific).

Enzymatic Digestions

Protein de-N-Glycosylation

N-glycans were removed using peptide N-glycosidase F from *Elizabethkingia meningoseptica* (PNGase F, 2U, Roche Diagnostics, Mannheim, Germany). IgM (50 μg) from both anti-MAG and MGUS groups was digested overnight at 37°C. Then, enzyme and N-glycans were cleaned by ultrafiltration using Amicon Ultra-100 K centrifugal filter devices (15 minutes, 4,500 g, 6 times). Each IgM extract before and after enzymatic treatment was stored at −80°C until further analysis.

Protein Desialylation

Desialylation was performed using neuraminidase from *Clostridium perfringens* (3.2.1.18; Sigma-Aldrich), which cleaves terminal sialic acid residues that are α2,3-linked, α2,6-linked, or α2,8-linked to glycoproteins. First, neuraminidase was diluted to 1 U/mL with pure water. As previously described,[26,27] 100 μL of serum was added to 100 μL of 0.1 M sodium acetate buffer pH 5, 25 μL of bovine serum albumin 1% (BSA, Sigma-Aldrich, St Louis, US) solution, and 25 μL of the enzyme solution. Enzyme removal and buffer exchange were performed as described above.

Under similar experimental conditions, but without enzyme, we confirm that overnight incubation at 37°C neither degrades IgM nor impairs its binding to MAG (data not shown). Enzymatic digestion efficacy was validated using polyacrylamide gel (NuPAGE 4–12% Bis-Tris gel,

Table Demographic and Clinical Features of the Cohort

	Anti-MAG group	MGUS group	HDs
Age (y)	73.5 (57–91)	75 (58–94)	35(27–46)
Sex (H/F)	8/9	4/4	3/3
Sensory deficit (n)	17	/	/
Motor deficit (n)	14	/	/
Tremor (n)	4	/	/
Ataxia (n)	8	/	/
RODS	80 (47–80)	/	/
ONLS	4 (0–9)	/	/
Demyelinating (n)	17	/	/
Waldenström macroglobulinemia (n)	5	/	/
Serum IgM concentration (g/L)	4.7 (1.4–10.9)	2.7 (1.3–10.6)	1.2(0.9–1.5)

Abbreviations: MGUS = monoclonal gammopathy of undetermined significance; ONLS = Overall Neuropathy Limitation Scale; RODS = Rasch-Built Overall Disability Scale.
RODS and ONLS scores were measured for 8 and 12 patients, respectively, at the time of sampling. Age, RODS score, ONLS score, and serum IgM concentration were presented as median (min-max) values.

Invitrogen) in denaturing conditions. After migration, proteins were stained with Coomassie blue (Sigma-Aldrich, eFigure 1). Deglycosylated IgM and desialylated IgM were used alternatively in the following experiments, depending on the previously established roles of sialyl residues or the overall N-glycans.

MAG Binding

Binding to MAG was assessed before and after IgM deglycosylation/desialylation (PNGase F or neuraminidase treatment) using an enzyme-linked immunosorbent assay (ELISA) (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer's instructions. Results were expressed in Bühlmann titer units (BTU), and a cutoff value of 1,000 BTU and upper value of 70,000 BTU were set.

Macrophage Stimulation and Cytokine Quantification

Macrophage stimulation was performed as previously described.[28] In brief, peripheral blood mononuclear cells were isolated from blood samples of HDs, by density gradient centrifugation over Ficoll-Paque (Eurobio). Monocytes for macrophage differentiation were isolated by plastic adherence. Then, cells were counted, seeded in 96-well *U*-bottom plates at a density of 5.10^4 cells/well, and cultured in RPMI-10% fetal calf serum (FCS) in the presence of 100 ng/mL of human Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) (Miltenyi Biotec) in a total volume of 100 µL. At day 6, macrophages were stimulated with 0.05 µg/µL of both glycosylated and deglycosylated IgM. After 24 hours, supernatants were harvested and stored at −80°C until analysis. Cytokine concentrations in the supernatant were measured using the CorPlex Human Cytokine 10-plex Panel 1 (IL-1β, IL-4, IL-5, IL-6, IL-8 [CXCL-8], IL-10, IL-12, IL-22, IFN-ϒ, and TNF-α) immunoassay on the Simoa Planar Technology Imaging and Detection System (Quanterix). The working dilution for all cytokines except IL-8 was 1:5 in diluent buffer. Because the IL-8 concentrations were all above the upper limit of quantification, IL-8 was measured using ELISA (Thermo Fisher, #88-8086-88) at working dilutions of 1:500 and 1:1,000. The lower limits of quantification (pg/mL) were as follows: IL-1β: 0.01, IL-4: 0.04, IL-5: 0.01, IL-6: 0.04, IL-8: 3.9, IL-10: 0.1, IL-12: 0.01, IL-22: 0.01, IFN-ϒ: 0.007, and TNF-α: 0.06. Each assay was duplicated.

N-Glycan Analysis of IgM By Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

IgM N-glycan profiles were studied by MALDI-TOF MS essentially as described previously.[29] In brief, IgM samples (10 µL of 0.1–1.0 mg/mL solutions) were diluted in 100 mM sodium phosphate buffer (PBS, pH 7.4) and 100 mM dithiothreitol solutions (final concentrations 20 mM and 10 mM, respectively, in a total volume of 49 µL) and then heated at 95°C for 5 minutes for protein denaturation. Protein de-N-glycosylation was performed after addition of PNGase F (2U, Roche Diagnostics, Mannheim, Germany) and an overnight incubation at 37°C. After acidification, proteins were precipitated using ice-cold ethanol for 1 hour at −20°C. Released N-glycans were further purified using porous graphitic carbon solid-phase extraction cartridges (Thermo Scientific, les Ulis, France) and subsequently permethylated. Permethylated N-glycans were analyzed using an UltrafleXtreme MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany) operating in the reflectron positive-ion mode and using 2,5-dihydroxybenzoic acid as matrix (10 mg/mL in 50% methanol containing 10 mM sodium acetate). MALDI-TOF mass spectra were internally calibrated and further processed using GlycoWorkBench software[30] for structure assignments and Flex Analysis software for peak integration (Bruker Daltonics). Abundances of the different N-glycan structures were reported as relative percentages to the total amount of annotated N-glycans.

C1q, DC-SIGN, and Fcα/µR Binding

Microtiter wells (Maxisorb, Nunc, Kamstrup, Denmark) were coated with 0.5 µg of native human C1q complement (Sigma-Aldrich, 204876-1mg) and recombinant DC-SIGN and recombinant Fcα/µR (Bio-Techne, Minneapolis, US) in 100 µL of PBS (pH 7.4). After incubation at 4°C, residual protein-binding sites were blocked by the addition of 200 µL of phosphate buffer saline-bovine serum albumine 3% (PBS-BSA) for 1 hour at room temperature. Initial concentrations of 50 µg/mL of purified IgM were used for DC-SIGN and Fcα/µR. Samples were subjected to serial 1:2 dilutions and incubated with C1q for 120 minutes at 4°C. After washing, HRP-labeled goat anti-human IgM (µ-chain–specific, A0420-Sigma-St Louis US, used at 1:1,000) was added for 60 minutes at room temperature, followed by tetramethylbenzidine (TMB) substrate (Thermo Fisher, US). Absorbance was measured at 450 nm. Control wells were incubated with secondary antibody only. For each sample, a corrected optical density (OD) was calculated (OD of the sample minus OD of control).

Statistical Analysis

Nonparametric and parametric tests were used including the Wilcoxon test to compare paired values, a Mann-Whitney test to compare 2 independent groups, and a Kruskal-Wallis test or Tukey test for multiple comparisons. The Spearman correlation test was used to measure the correlation between 2 variables. Significant p values are indicated as described: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Statistical analyses were performed using GraphPad Prism software V9 (GraphPad, San Diego) and *R* studio software (version 2026.06.1).

Standard Protocol Approvals, Registrations, and Patient Consents

The study has been approved by the ethics committees of the 2 participating centers (agreement PADS 22–362, CPP Ile de France VI). Patients gave written informed consent for research use of their biological samples.

Data Availability

All relevant data are available within the article and the supplementary materials. Anonymized data will be shared on reasonable request from any qualified investigator to the corresponding author.

Results

A Unique IgM N-Glycan Signature in Anti-MAG Neuropathy

To decipher IgM N-glycome in patients with anti-MAG neuropathy, IgM N-glycans were analyzed by MALDI-TOF MS after conventional PNGase F treatment and compared with IgM N-glycans from the MGUS group and healthy individuals (Figure 1A). As expected, we observed a wide variety of complex mannosylated N-glycans in healthy individuals,[5] but also in both MGUS and anti-MAG groups. Overall, we detected 14 major IgM N-glycan structures (with relative abundance >1%) in the 3 groups (Figure 1B). N-glycan structures along with their respective relative intensities are presented in eTable 1 and eFigure 2. To analyze general trends in the IgM N-glycan profiles across the 3 groups, we performed a principal component analysis, which emphasized significant N-glycan profile differences (R^2 = 0.45, $p = 0.001$) between patients with anti-MAG neuropathy, those with MGUS, and HDs (Figure 1C). Strikingly, 1 IgM N-glycan (peak 12, fucosylated biantennary monosialylated N-glycan with a bisecting N-acetyl glucosamine residue) significantly contributed to the observed clustering and appeared as the most abundant structure in the anti-MAG N-glycan profile, accounting for approximately half of the total N-glycan pool (Figure 1D, median [min-max] anti-MAG IgM 48.5 [33.3–53] vs MGUS IgM 27.3 [0.7–44], $p < 0.01$). In addition, the total amount of N-glycans with a bisecting GlcNAc moiety increased in anti-MAG IgM compared with IgM from MGUS and HD groups (Figure 1D, anti-MAG IgM 66.3 [47.6–70.9] vs MGUS IgM 37.1 [12.6–64.9] vs HD IgM 51.7 [34.7–63.6], $p < 0.01$ and $p = 0.05$, respectively). Third, mannosylation levels of anti-MAG IgM were intermediate between those of MGUS IgM and IgM from healthy controls (Figure 1D, anti-MAG IgM 23.4 [18.6–37.1] vs MGUS IgM 54.6 [19.6–75.1] vs HD IgM 17.25 [14.8–28.9], $p < 0.05$ and $p = 0.05$, respectively).

Taken together, these results indicate that patients with anti-MAG neuropathy display a distinct IgM N-glycosylation signature dominated by a fucosylated and monosialylated N-glycan with a bisecting GlcNAc.

IgM N-Glycosylation Influences MAG Binding

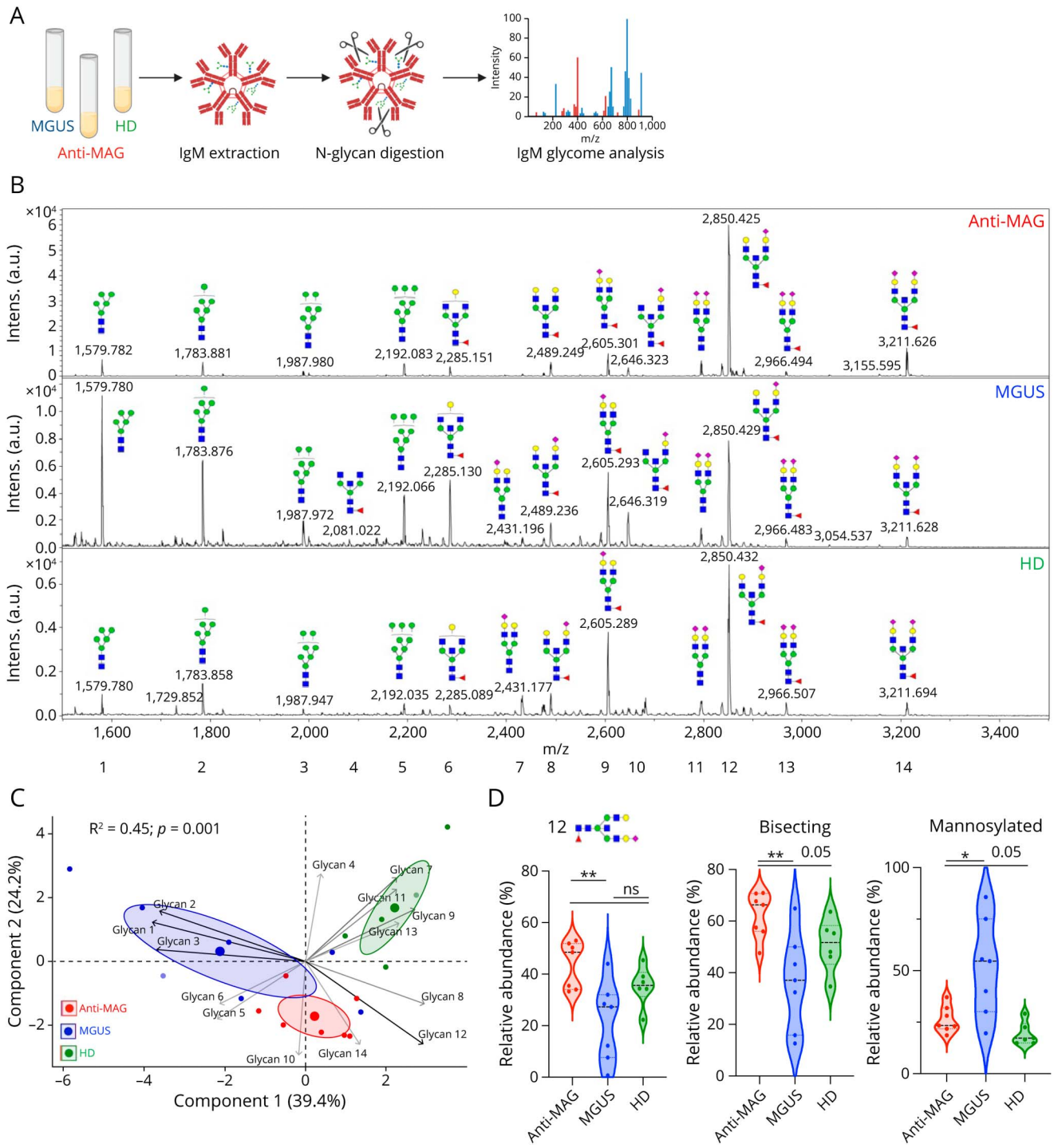
As a member of the SIGLEC family, MAG shows affinity for glycoproteins, especially with sialylated glycoproteins.[31] Thus, we postulated that specific anti-MAG N-glycans might affect MAG binding. Hence, we measured MAG binding of anti-MAG IgM before and after deglycosylation. As shown in Figure 2A, enzymatic removal of anti-MAG IgM N-glycans resulted in a clear drop in their interaction with the MAG antigen (average % of decrease 58.3 ± 18.8, $p < 0.01$), confirming contribution of N-glycans in the binding. Of note, 11 of 17 samples (64.7%) showed a reduction in anti-MAG titers after deglycosylation while 6 samples remained above the measurement range (i.e., >70,000 BTU). Because the monosialylated N-glycan (peak 12, Figure 1B) predominated among the anti-MAG IgM N-glycome, and because the SIGLEC family interacts preferentially with sialylated glycoproteins, we further explored the role of IgM sialylation in MAG binding. In line with the latter results, we observed a 27% decrease in anti-MAG IgM binding after desialylation (average % of decrease 27 ± 13%, $p < 0.001$, Figure 2B). After desialylation, 15 of 17 samples (88%) displayed significant reduction in MAG binding. Three samples, exceeding the upper limit (i.e., 70,000 BTU), could not be evaluated.

Taken together, the latter observations show that the specific IgM N-glycans may be associated with anti-MAG neuropathy because of a functional impact on IgM interaction with MAG.

Particular Anti-MAG IgM N-Glycosylation Enhances Binding to C1q

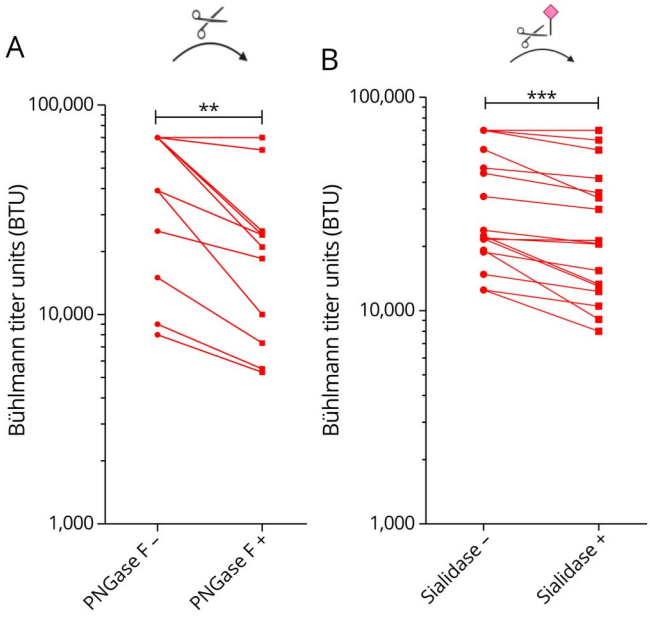
Anti-MAG neuropathy is associated with deposits of IgM paraprotein and complement on the myelin sheaths,[32,33] likely contributing to myelin degradation and axonal degeneration.[34,35] Moreover, previous reports demonstrated that IgG and IgM N-glycans interact with C1q[36] and modulate the classical complement pathway activation.[9,37] We, therefore, sought to interrogate the ability of IgM binding to C1q among the 3 groups. We adapted an ELISA assay by coating plates with native human complement C1q followed by incubation with increasing concentrations of purified IgM (Figure 3A). Of interest, anti-MAG IgM showed higher binding to C1q compared with MGUS IgM and HD IgM (Figure 3A), suggesting that anti-MAG IgM N-glycosylation may influence binding to C1q. Moreover, these differences are more pronounced at higher IgM concentrations. At a concentration of 1.6 μg/mL, we observed a slight increase in anti-MAG IgM binding to C1q, in contrast to HD IgM and MGUS IgM, which do not have the ability to bind to C1q (anti-MAG IgM binding to C1q 0.24 [0.18–0.66] vs MGUS IgM 0.15 [0.09–0.21] $p < 0.0001$ vs HD IgM 0.2 [0.16–0.27] $p = 0.06$, Figure 3B). At a concentration of 50 μg/mL, which is far below serum IgM concentration in anti-MAG neuropathy (Table), anti-MAG IgM was found to be three to four times more potent in C1q binding than MGUS IgM and HD IgM (median [25th and 75th percentiles]: anti-MAG IgM 1.51 [1.16–2.4] vs MGUS IgM 0.4 [0.20–0.89] $p < 0.0001$ vs HD IgM 0.54 [0.40–0.71] $p < 0.001$, Figure 3C). Of note, no correlation was found between anti-MAG titer and C1q binding (r_s = −0.19 [-0.72-0.48], $p = 0.6$). These results suggest that the N-glycosylation pattern may affect C1q binding. There is no doubt that sialylation of IgG significantly can affect complement-dependent cytotoxicity and IgG binding to C1q.[38] Emerging data also suggest that sialidase digestion of IgM reduces complement deposition.[9] To further

Figure 1 Anti-MAG IgM Exhibited a Distinct N-Glycan Signature



(A) Experimental procedure. (B) Representative MALDI-TOF mass spectra of permethylated PNGase F–released N-glycans from anti-MAG, MGUS, and healthy donor IgM samples. Measurements were performed in the positive-ion mode, and all ions are present in sodiated form. (C) PCA of the 14 major N-glycans measured in anti-MAG patients (n = 7), those with MGUS (n = 7), and controls (n = 6). Ellipses represent 50% CI of patient distribution in each group. PCA significance was determined using the Adonis test. (D) Relative abundances of N-glycan (peak 12), bisecting and mannosylated N-glycans in the 3 groups. Bisecting refers to any glycans with a bisecting GlcNAc moiety (4; 6; 8; 10; 12; 14), and mannosylated refers to any glycans with a terminal mannose (1; 2; 3; 5). Nomenclature of sugars: green circles, mannose; yellow circles, galactose; blue squares, N-acetyl glucosamine; red triangles, fucose; purple diamonds, sialic acid. Results are shown as median and 25th and 75th percentiles. Data were analyzed for significance using a Tukey multiple comparison test. *$p < 0.05$; **$p < 0.001$. Anti-MAG = anti–myelin-associated glycoprotein; MALDI-TOF = matrix-assisted laser desorption/ionization time-of-flight; MGUS = monoclonal gammopathy of undetermined; significance; ns = not significant

Figure 2 IgM N-Glycosylation Modulates Anti-MAG Activity



MAG binding was tested before and after (A) total N-deglycosylation using PNGase F and (B) desialylation using sialidase (n = 17). BTU = BÜHLMANN titer units. The top line corresponds to samples with anti-MAG activity exceeding 70000 BTU (converted to semi-log units), i.e., out of the assay measuring range before and after deglycosylation (n = 6) and desialylation (n = 3). MAG ELISA before and after deglycosylation was performed in the same test while MAG ELISA before and after desialylation was performed the following day. All tests were performed in duplicate. Differences were analyzed using a Wilcoxon test (**$p < 0.01$; ***$p < 0.001$). Anti-MAG = anti–myelin-associated glycoprotein

explore the role of sialic acid residues in anti-MAG IgM binding to C1q, anti-MAG IgM samples were digested with neuraminidase and compared with the paired undigested samples. Neuraminidase treatment significantly reduced anti-MAG IgM binding to C1q (average % of decrease 40.0 ± 19.9, $p < 0.05$; Figure 3D). Taken together, the propensity of anti-MAG IgM to bind C1q seems sialic acid dependent.

Induction of Proinflammatory Cytokines by Anti-MAG IgM Is Dependent on N-Glycosylation

Anti-MAG neuropathy has been associated with increased serum levels of IL-6.[39] Moreover, serum samples from patients with anti-MAG neuropathy significantly upregulated the genes associated with TNF-α, NF-kB, and downstream pathway molecules of NF-kB.[40] To determine whether the anti-MAG IgM N-glycosylation pattern influences cytokine production by macrophages, we incubated anti-MAG IgM and MGUS IgM with monocyte-derived macrophages and measured cytokine levels in supernatants (Figure 4A). Like endothelial cells,[40] macrophages showed increased TNF-α production after stimulation with anti-MAG IgM compared with MGUS IgM (median [min-max] 155 [61–702] vs 72 [30–152], $p < 0.01$, Figure 4B). In addition to TNF-α, anti-MAG IgM demonstrated a broad proinflammatory effect on macrophages, leading to a 3–13-fold increase in IL-8, IL-1β, IL-6, and IFN-γ levels, compared with MGUS IgM (anti-MAG IgM vs MGUS IgM: IL-8 $p < 0.01$, IL-1β $p < 0.001$, IL-6 $p < 0.01$, IFN-γ $p < 0.001$, Figure 4B and eFigure 3A). Conversely, similar levels of IL-4, IL-5, IL-10, IL-12, and IL-22 were measured in macrophage culture supernatants after incubation with either anti-MAG IgM or MGUS IgM (eFigure 3B). Because alteration in IgG whole N-glycosylation correlated with proinflammatory potential,[41] we explored whether anti-MAG IgM N-glycosylation may contribute to this effect on macrophages. We, therefore, incubated macrophages with IgM before and after N-deglycosylation with PNGase F. Strikingly, deglycosylated anti-MAG IgM had a significantly reduced capacity to induce IL-8, TNF-α, and IFN-γ compared with native IgM (native IgM vs deglycosylated anti-MAG IgM: IL-8 and TNF-α $p < 0.01$; IFN-γ $p < 0.05$, Figure 4B). No differences were observed for IL-1β and IL-6 (eFigure 3A). Moreover, native IgM and deglycosylated MGUS IgM revealed a similar cytokine production profile (Figure 4B and eFigure 3A).

To gain further insights into the immunologic remodeling of monocyte-derived macrophages that is induced with anti-MAG IgM, we analyzed, using ELISA, the interaction between anti-MAG IgM and its receptors such as Fcα/μ receptor and DC-SIGN that are both expressed on macrophages.[42] We found that anti-MAG IgM bound more strongly Fcα/μ receptor and DC-SIGN compared with MGUS IgM ($p < 0.05$ and $p = 0.07$, eFigure 4), which could in turn trigger macrophage activation and cytokine production.

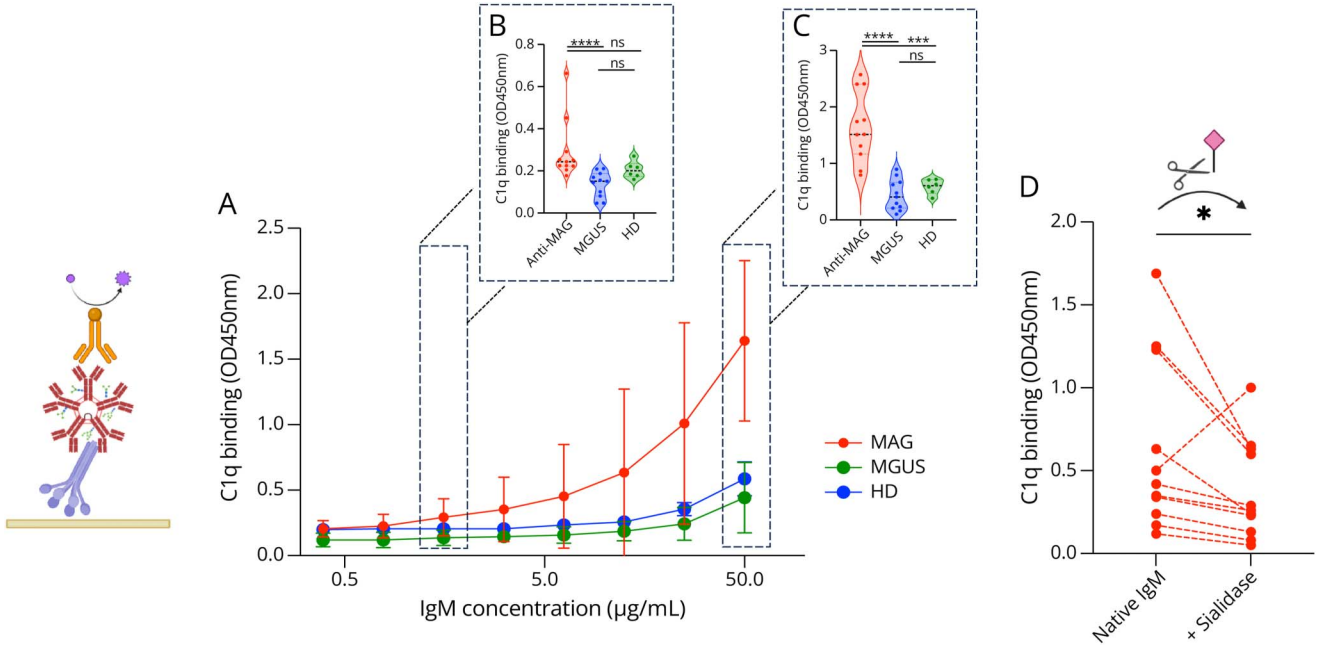
Taken together, these results support the notion that N-glycans on anti-MAG IgM might contribute to shifting macrophage cytokine production toward a proinflammatory profile.

Discussion

Anti-MAG IgM antibodies are considered pathogenic because of their complement-activating and proinflammatory properties.[43] However, it remains unclear whether anti-MAG antibodies differ from classical IgM and how they acquire such an unusual pathogenic potential. In this study, we present the first characterization of the N-glycosylation profile of anti-MAG IgM and provide insights into the role of N-glycans in anti-MAG IgM pathogenicity. Thus, we found that anti-MAG IgM N-glycans play a crucial role in MAG binding and enhance interaction with C1q. We report that proinflammatory cytokines such as TNF-α, IL-8, IL-1β, IL-6, and IFN-γ were significantly upregulated after macrophage exposure to anti-MAG IgM.

Our results reinforced the role of complement activation in anti-MAG neuropathy as we demonstrated that anti-MAG IgM strongly binds to C1q. Anti-MAG IgM has been identified as the primary mediator of myelin alterations, with complement acting as the effector[44] and the myelinolytic effect requiring active human complement.[21] In our study, anti-

Figure 3 Anti-MAG N-Glycosylation Signature Enhances IgM Binding to C1q



(A) IgM binding to C1q was measured by ELISA at indicated concentrations. Red, green, and blue curves represent anti-MAG, MGUS, and HD samples (anti-MAG, n = 11; MGUS, n = 11; HD, n = 6). (B and C) Binding to 0.5 µg of native human C1q complement of 1.6 µg/mL and 50 µg/mL of native IgM. Data are presented as median and 25th and 75th percentiles from 3 technical replicates. (D) Anti-MAG IgM (50 µg/mL) binding to C1q before and after digestion by sialidase. Data are generated from 2 technical replicates. Asterisks indicate significance with a Mann-Whitney test (B and C) and a Wilcoxon test (D) (*$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$). Anti-MAG = anti–myelin-associated glycoprotein; MGUS = monoclonal gammopathy of undetermined significance.

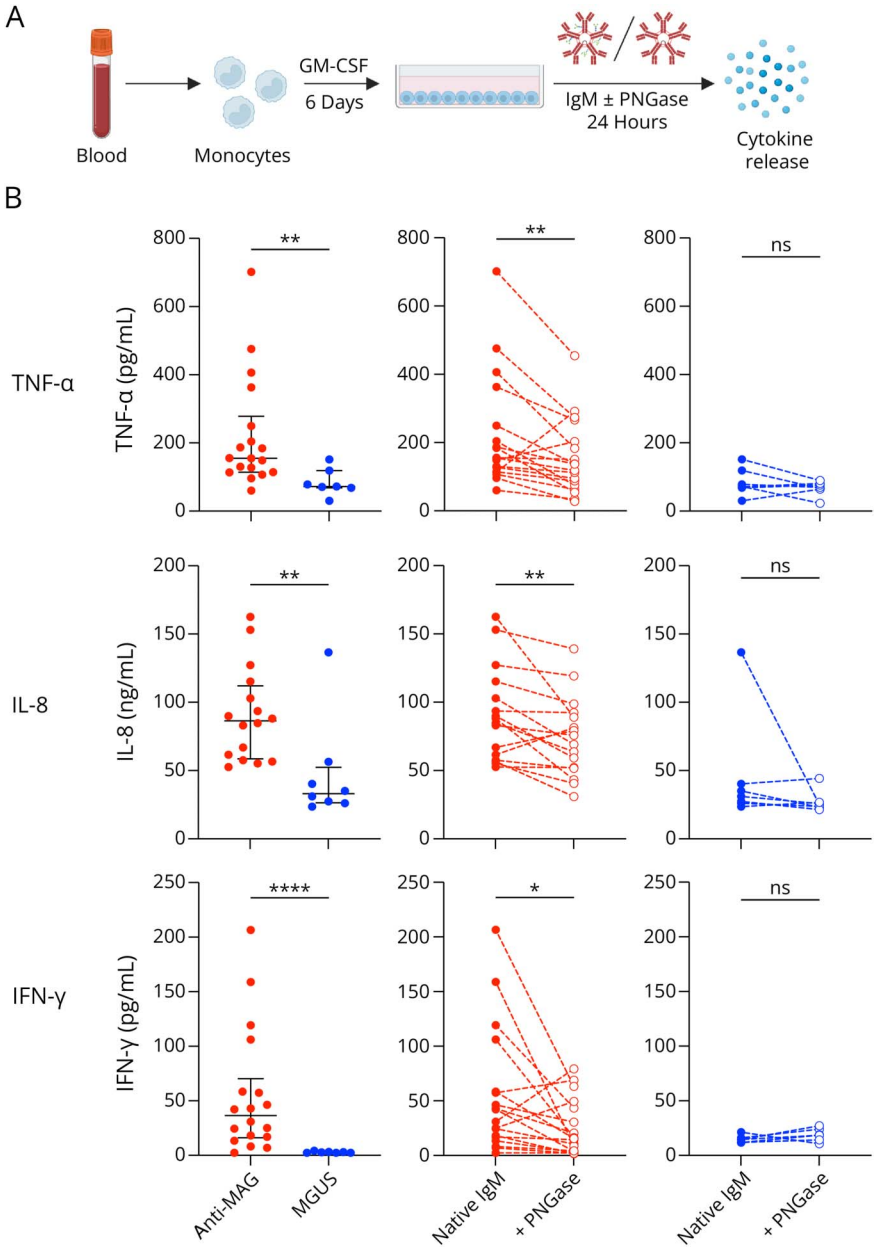
MAG binding to C1q does not correlate with the level of anti-MAG activity or the amount of IgM, suggesting that it rather depends on the IgM protein structure and N-glycosylation pattern of its heavy chain, as already shown by other authors.[45] While correlation between anti-MAG titers and complement activation remains conflicting in the literature,[46] which may be explained by the cohort sizes and the assay performances, there is a broad consensus that the complement-activating capacity of anti-MAG is crucial and could serve as a biomarker for the disease. The ability of anti-MAG IgM to bind to C1q, easily tested by ELISA as set up in our study, could serve as a valuable biological marker for disease progression, given that a decrease in serum complement activity is not a sensitive indicator of this localized intratissue complement activation.[39] The significant variability observed among our patients may reflect an ongoing state of active neurologic involvement. Nevertheless, although previous studies have established a correlation between C1q binding and complement-dependent cytolysis,[47] we acknowledge that additional experiments, such as complement-dependent lysis or complement deposition assays, would be required to assess the functional impact of the enhanced C1q–anti-MAG IgM interaction.

We also observed that anti-MAG IgM sialylation favors C1q binding. In line with our results, previous studies have shown that complement deposition increases in correlation with the sialylation of IgM.[48] Of interest, anti-MAG sialylation is not only involved in interactions with complement but also enhances the binding of the monoclonal IgM to its target. Indeed, MAG has a strong affinity for alpha2,3-sialic acid and, to a lesser extent, alpha2,6-sialic acid.[14] It interacts with myelin components such as gangliosides that share this molecular structure initiating a signaling cascade essential for the maintenance and survival of myelinated axons. One could hypothesize that anti-MAG sialylation allows them to replace natural "binding partners"[14] of MAG such as gangliosides, thereby blocking this signaling cascade and contributing to the clinical phenotype.

In this study, we report that proinflammatory cytokines, including TNF-α, IL-8, IL-1β, IL-6, and IFN-γ, were significantly upregulated after macrophage exposure to anti-MAG IgM. Notably, we did not observe an increase in IL-10, which contrasts with previous findings.[49,39] This absence of IL-10 overexpression may be attributed to the polarization of macrophages toward the proinflammatory M1 phenotype, driven by GM-CSF stimulation, thereby bypassing the anti-inflammatory M2 phenotype responsible for producing T-regulatory cytokines such as IL-10.

The observed cytokine overexpression was specific to anti-MAG IgM and unrelated to the paraproteinemic status of patients, because MGUS IgM did not induce a similar effect. Our findings suggest that N-glycosylation modulates the effector response of IgM, with specific N-glycan structures on

Figure 4 Anti-MAG N-Glycosylation Signature Promotes Proinflammatory Cytokine Release



(A) Experimental procedure. (B) Cytokines were measured in culture supernatants after 24 hours of incubation with 50 µg/mL of native anti-MAG IgM and MGUS IgM (red and blue points, left panel). Cytokines were compared in culture supernatants after 24 hours of incubation with anti-MAG (middle panel in red) and MGUS (right panel in blue) IgM (50 µg/mL) before and after N-deglycosylation with PNGase (colored and empty circles, respectively). Anti-MAG group, n = 16; MGUS group, n = 8. In the left panel, data are presented as median and 25th and 75th percentiles. Data were generated from 3 technical replicates. Asterisks indicate significance with a Mann-Whitney test (left panel) and a Wilcoxon test (middle and right panel) (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Anti-MAG = anti–myelin-associated glycoprotein; MGUS = monoclonal gammopathy of undetermined significance.

anti-MAG IgM contributing to its pathogenicity. Cytokine secretion patterns were determined mainly by the N-glycan structures present on anti-MAG IgM because macrophage stimulation was performed using the same cell population exposed to identical amounts of both N-glycosylated and deglycosylated IgM. N-glycan–dependent interactions have been identified among various proinflammatory cytokines, exhibiting synergistic effects that increase blood-nerve barrier permeability and contribute to nerve damage.[50]

Of interest, the effects of IgM sialylation on macrophage activation differed from previous findings, where sialylation was shown to inhibit T-cell activation.[10] Regarding relative intensity, sialylated residues accounted for 45.6% of the total N-glycan pool in MGUS IgM and 71.4% in anti-MAG IgM. While this percentage was lower in MGUS IgM, it remained significant but insufficient to trigger macrophage activation. Furthermore, even after complete deglycosylation, anti-MAG IgM retained proinflammatory properties, albeit with reduced intensity, particularly for IL-8, TNF-α, and IFN-γ (Figure 4B, middle panel). This suggests a synergistic effect between the intrinsic activity of anti-MAG IgM and its glycosylation profile in driving the proinflammatory response. The proinflammatory potential of anti-MAG IgM seems to be influenced by multiple factors, including sialylation levels, the specific sugar moieties present, the intrinsic activity of IgM,

the type of cell involved, and the nature of the Fc receptor engaged. Various Fc receptors, including the polymeric immunoglobulin receptor (pIgR), Fcα/μR, and FcμR, contribute to the inflammatory effects of IgM.[42] These receptors undergo rapid turnover on the cell membrane and can be modulated by IgM binding and its serum concentration.[e1] We hypothesize that persistent monoclonal IgM expression may shift signaling from inhibitory receptors on T lymphocytes toward activating signals on macrophages, thereby promoting inflammatory effector responses. In addition, our results indicate that anti-MAG IgM binds more strongly to Fcα/μR than to DC-SIGN. However, the involvement of other receptors, such as pIgR, as well as other cells expressing these receptors in vivo, cannot be excluded. Further studies are necessary to elucidate how anti-MAG IgM and its N-glycans are internalized and interact with intracellular components to regulate the immune effector response.

Our findings suggest that IL-8 upregulation, mediated by anti-MAG IgM, was N-glycan dependent and associated with the expression of proinflammatory cytokines IL-1 and TNF-α. These cytokines significantly increase IL-8 transcription and the mRNA stability.[e2,e3] IL-8 plays a critical role in anti-MAG neuropathy by activating macrophages and recruiting monocytes to the nerve. Studies have reported overexpression of this chemokine in patients with polyneuropathies, including anti-MAG neuropathy,[e4] and in animal models of chronic nerve injury.[e5] Although rituximab reduces B cells producing anti-MAG antibodies,[19,e6] it does not affect antibody activity. One can hypothesize that combining rituximab with an indirect IL-8 synthesis inhibitor targeting bruton tyrosine kinase (BKT) might improve treatment outcomes. This previously described approach[e7,e8] blocks the inflammatory processes that damage the blood-nerve barrier and reduces the accumulation of antibodies in myelin sheaths. However, involvement of IL-8 in this mechanism was not explored. Three studies have shown that IL-1 and TNF-α regulate the expression of the IL-8 gene.[e2,e3,e9] Furthermore, BKT has been involved in the induction of IL-1 and TNF-α,[e10] Therefore, it is reasonable to assume that using an anti-BKT would indirectly modulate IL-8 expression. Moreover, owing to the significant IgM binding to C1q, certain drugs, such as sutimlimab, which specifically target C1 of the complement protein in the classical pathway, could offer a promising therapeutic approach. These therapeutic options require the accumulation of additional in vitro and ex vivo data before considering large clinical trials and real-world application.

The number of controls is relatively low compared with the patients. Although the sample size was limited in the control group, significant differences were observed regarding binding capacities of C1q, Fcα/μ, and DC-SIGN ($p < 0.001$, $p < 0.05$, and $p = 0.06$, respectively), somewhat reinforcing the relevance of our observation. In addition, while the MGUS group is age-matched, the HDs are younger than the anti-MAG patients, which could influence the glycosylation profile. Given that patients with MGUS share both age and IgM monoclonality with anti-MAG patients, they serve as the most appropriate control group. Further research is needed to clarify how age alone affects IgM glycosylation, independent of disease.

Between patients, we observed that anti-MAG IgM exhibits heterogeneous activity toward both the HNK1 epitope and MAG natural binding partners, such as gangliosides, potentially disrupting this signaling pathway. It remains unclear whether glycosylation profiles would define distinct clinical phenotypes and to which extent they are influenced by treatments. It also remains unclear whether the modifications observed would occur early enough to influence the clinical progression of neuropathy. Additional research, especially in newly diagnosed and untreated patients, is needed to explore this further.

This study underlines the impact of N-glycans attached to anti-MAG IgM on the pathophysiology of this antibody, emphasizing the role of glycosylation that increases the proinflammatory effects of IgM. Our findings revealed 2 potential avenues for further biological investigations: the ability of IgM to bind to C1q and the role of sialylated residues in anti-MAG activity. These 2 aspects could improve the correlation between biological results and clinical outcomes, but they require evaluation in newly diagnosed patients. Given the challenges of treating this neuropathy, this study provides an immunologic foundation for expanding therapeutic options, suggesting that drugs targeting the IL-8/CXCL-8 pathway, or the classical complement pathway could serve as complementary treatments alongside B-cell depletion.

Author Contributions

J. Neil: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. F. Fenaille: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. A. Bruneel: drafting/revision of the manuscript for content, including medical writing for content. T. Stojkovic: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. S. Cholet: analysis or interpretation of data. E. Delmont: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. P. Ober: analysis or interpretation of data. A. Raynor: analysis or interpretation of data. Q. Amiot: analysis or interpretation of data. K. Dorgham: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. K. Viala: drafting/revision of the manuscript for content, including medical writing for content. P. Ghillani-Dalbin: drafting/revision of the manuscript for content, including medical writing for content. G. Gorochov: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or

interpretation of data. D. Sterlin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology® Neuroimmunology & Neuroinflammation* January 13, 2025. Accepted in final form May 9, 2025. Submitted and externally peer reviewed. The handling editor was Marinos C. Dalakas, MD, FAAN.

References

1. Goulabchand R, Batteux F, Guilpain P. (Glycosylation of autoantibodies in autoimmunes diseases). *LA Revue de Médecine Interne*. 2013;34(12):746-753. doi:10.1016/j.revmed.2013.09.005
2. Cobb BA. The history of IgG glycosylation and where we are now. *Glycobiology*. 2020;30(4):202-213. doi:10.1093/glycob/cwz065
3. Vattepu R, Sneed SL, Anthony RM. Sialylation as an important regulator of antibody function. *Front Immunol*. 2022;13:818736. doi:10.3389/fimmu.2022.818736
4. Chandler KB, Mehta N, Leon DR, Suscovich TJ, Alter G, Costello CE. Multi-isotype glycoproteomic characterization of serum antibody heavy chains reveals isotype- and subclass-specific N-glycosylation profiles. *Mol Cel Proteomics*. 2019;18(4):686-703. doi:10.1074/mcp.RA118.001185
5. Arnold JN, Wormald MR, Suter DM, et al. Human serum IgM glycosylation: identification of glycoforms that can bind to mannan-binding lectin. *J Biol Chem*. 2005;280(32):29080-29087. doi:10.1074/jbc.M504528200
6. Torii T, Kanemitsu K, Hagiwara A. Sialic acid level is significantly elevated in IgM enriched protein fraction in sera of cancer patients. *J Immunoassay Immunochem*. 2017;38(2):127-139. doi:10.1080/15321819.2016.1224973
7. Vreeker GCM, Vangangelt KMH, Bladergroen MR, et al. Serum N-glycan profiles differ for various breast cancer subtypes. *Glycoconj J*. 2021;38(3):387-395. doi:10.1007/s10719-021-10001-3
8. Haslund-Gourley BS, Hou J, Woloszczuk K, et al. Host glycosylation of immunoglobulins impairs the immune response to acute Lyme disease. *EBioMedicine*. 2024;100:104979. doi:10.1016/j.ebiom.2024.104979
9. Haslund-Gourley BS, Woloszczuk K, Hou J, et al. IgM N-glycosylation correlates with COVID-19 severity and rate of complement deposition. *Nat Commun*. 2024;15(1):404. doi:10.1038/s41467-023-44211-0
10. Colucci M, Stöckmann H, Butera A, et al. Sialylation of N-linked glycans influences the immunomodulatory effects of IgM on T cells. *J Immunol*. 2015;194(1):151-157. doi:10.4049/jimmunol.1402025
11. Magy L, Kaboré R, Mathis S, et al. Heterogeneity of polyneuropathy associated with anti-MAG antibodies. *J Immunol Res*. 2015;2015:450391. doi:10.1155/2015/450391
12. Svahn J, Petiot P, Antoine JC, et al. Anti-MAG antibodies in 202 patients: clinicopathological and therapeutic features. *J Neurol Neurosurg Psychiatry*. 2018;89(5):499-505. doi:10.1136/jnnp-2017-316715
13. Min YG, Visentin A, Briani C, Rajabally YA. Neuropathy with anti-myelin-associated glycoprotein antibodies: update on diagnosis, pathophysiology and management. *J Neurol Neurosurg Psychiatry*. 2025;96(4):340-349. doi:10.1136/jnnp-2024-334678
14. Quarles RH. Myelin-associated glycoprotein (MAG): past, present and beyond. *J Neurochem*. 2007;100(6):1431-1448. doi:10.1111/j.1471-4159.2006.04319.x
15. Ilyas AA, Quarles RH, MacIntosh TD, et al. IgM in a human neuropathy related to paraproteinemia binds to a carbohydrate determinant in the myelin-associated glycoprotein and to a ganglioside. *Proc Natl Acad Sci U S A*. 1984;81(4):1225-1229. doi:10.1073/pnas.81.4.1225
16. McGarry RC, Helfand SL, Quarles RH, Roder JC. Recognition of myelin-associated glycoprotein by the monoclonal antibody HNK-1. *Nature*. 1983;306(5941):376-378. doi:10.1038/306376a0
17. Ilyas AA, Quarles RH, Dalakas MC, Brady RO. Polyneuropathy with monoclonal gammopathy: glycolipids are frequently antigens for IgM paraproteins. *Proc Natl Acad Sci U S A*. 1985;82(19):6697-6700. doi:10.1073/pnas.82.19.6697
18. Ilyas AA, Li SC, Chou DK, et al. Gangliosides GM2, IV4GalNAcGM1b, and IV4GalNAcGC1a as antigens for monoclonal immunoglobulin M in neuropathy associated with gammopathy. *J Biol Chem*. 1988;263(9):4369-4373. doi:10.1016/s0021-9258(18)68935-5
19. Dalakas MC. Advances in the diagnosis, immunopathogenesis and therapies of IgM-anti-MAG antibody-mediated neuropathies. *Ther Adv Neurol Disord*. 2018;11:1756285617746640. doi:10.1177/1756285617746640
20. Dalakas MC, Quarles RH. Autoimmune ataxic neuropathies (sensory ganglionopathies): are glycolipids the responsible autoantigens? *Ann Neurol*. 1996;39(4):419-422. doi:10.1002/ana.410390402
21. Hays AP, Latov N, Takatsu M, Sherman WH. Experimental demyelination of nerve induced by serum of patients with neuropathy and an anti-MAG IgM M-protein. *Neurology*. 1987;37(2):242-256. doi:10.1212/wnl.37.2.242
22. Willison HJ, Trapp BD, Bacher JD, Dalakas MC, Griffin JW, Quarles RH. Demyelination induced by intraneural injection of human antimyelin-associated glycoprotein antibodies. *Muscle Nerve*. 1988;11(11):1169-1176. doi:10.1002/mus.880111111
23. Van den Bergh PY, Hadden RD, Bouche P, et al. European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society - first revision. *Eur J Neurol*. 2010;17(3):356-363. doi:10.1111/j.1468-1331.2009.02930.x
24. Van den Bergh PYK, van Doorn PA, Hadden RDM, et al. European Academy of Neurology/Peripheral Nerve Society guideline on diagnosis and treatment of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint Task Force-Second revision. *Eur J Neurol*. 2021;28(11):3556-3583. doi:10.1111/ene.14959
25. Neil J, Choumet V, Beadon K, et al. Native versus deglycosylated IgM in anti-MAG neuropathy: correlation with clinical status - study of 10 cases. *J Neuroimmunol*. 2020;339:577094. doi:10.1016/j.jneuroim.2019.577094
26. Caslavska J, Lanz C, Burda P, Tobler M, Thormann W. Analysis of genetic variants of transferrin in human serum after desialylation by capillary zone electrophoresis and capillary isoelectric focusing. *J Sep Sci*. 2017;40(11):2488-2497. doi:10.1002/jssc.201700211
27. Sakhi S, Cholet S, Wehbi S, et al. MAN1B1-CDG: three new individuals and associated biochemical profiles. *Mol Genet Metab Rep*. 2021;28:100775. doi:10.1016/j.ymgmr.2021.100775
28. Sterlin D, Fadlallah J, Adams O, et al. Human IgA binds a diverse array of commensal bacteria. *J Exp Med*. 2020;217(3):e20181635. doi:10.1084/jem.20181635
29. Bruneel A, Cholet S, Drouin-Garraud V, et al. Complementarity of electrophoretic, mass spectrometric, and gene sequencing techniques for the diagnosis and characterization of congenital disorders of glycosylation. *Electrophoresis*. 2018;39(24):3123-3132. doi:10.1002/elps.201800021
30. Ceroni A, Maass K, Geyer H, Geyer R, Dell A, Haslam SM. GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res*. 2008;7(4):1650-1659. doi:10.1021/pr7008252
31. Schwardt O, Kelm S, Ernst B. SIGLEC-4 (MAG) antagonists: from the natural carbohydrate epitope to glycomimetics. *Top Curr Chem*. 2015;367:151-200. doi:10.1007/128_2013_498
32. Nobile-Orazio E. Antigenic determinants in IgM paraprotein-related neuropathies. *Clin Lymphoma Myeloma*. 2009;9(1):107-109. doi:10.3816/CLM.2009.n.029
33. Hays AP, Lee SS, Latov N. Immune reactive C3d on the surface of myelin sheaths in neuropathy. *J Neuroimmunol*. 1988;18(3):231-244. doi:10.1016/0165-5728(88)90101-4
34. Mead RJ, Singhrao SK, Neal JW, Lassmann H, Morgan BP. The membrane attack complex of complement causes severe demyelination associated with acute axonal injury. *J Immunol*. 2002;168(1):458-465. doi:10.4049/jimmunol.168.1.458
35. Stoll G, Schmidt B, Jander S, Toyka KV, Hartung HP. Presence of the terminal complement complex (C5b-9) precedes myelin degradation in immune-mediated demyelination of the rat peripheral nervous system. *Ann Neurol*. 1991;30(2):147-155. doi:10.1002/ana.410300205
36. Sharp TH, Boyle AL, Diebolder CA, Kros A, Koster AJ, Gros P. Insights into IgM-mediated complement activation based on in situ structures of IgM-C1-C4b. *Proc Natl Acad Sci U S A*. 2019;116(24):11900-11905. doi:10.1073/pnas.1901841116
37. Krištić J, Lauc G. The importance of IgG glycosylation-What did we learn after analyzing over 100,000 individuals. *Immunol Rev*. 2024;328(1):143-170. doi:10.1111/imr.13407
38. Trzos S, Link-Lenczowski P, Pocheć E. The role of N-glycosylation in B-cell biology and IgG activity. The aspects of autoimmunity and anti-inflammatory therapy. *Front Immunol*. 2023;14:1188838. doi:10.3389/fimmu.2023.1188838
39. Stork ACJ, Rijkers GT, Vlam L, et al. Serum cytokine patterns in immunoglobulin m monoclonal gammopathy-associated polyneuropathy. *Muscle Nerve*. 2019;59(6):694-698. doi:10.1002/mus.26462
40. Sato R, Shimizu F, Kuwahara M, et al. Autocrine TNF-alpha increases penetration of myelin-associated glycoprotein antibodies across the blood-nerve barrier in anti-MAG neuropathy. *Neurol Neuroimmunol Neuroinflamm*. 2023;10(3):e200086. doi:10.1212/NXI.0000000000200086
41. Gaifem J, Rodrigues CS, Petralia F, et al. A unique serum IgG glycosylation signature predicts development of Crohn's disease and is associated with pathogenic antibodies to mannose glycan. *Nat Immunol*. 2024;25(9):1692-1703. doi:10.1038/s41590-024-01916-8
42. Klimovich VB. IgM and its receptors: structural and functional aspects. *Biochemistry (Mosc)*. 2011;76(5):534-549. doi:10.1134/S0006297911050038
43. Lombardi R, Erne B, Lauria G, et al. IgM deposits on skin nerves in anti-myelin-associated glycoprotein neuropathy. *Ann Neurol*. 2005;57(2):180-187. doi:10.1002/ana.20364
44. Monaco S, Ferrari S, Bonetti B, et al. Experimental induction of myelin changes by anti-MAG antibodies and terminal complement complex. *J Neuropathol Exp Neurol*. 1995;54(1):96-104. doi:10.1097/00005072-199501000-00012
45. Miletic VD, Frank MM. Complement-immunoglobulin interactions. *Curr Opin Immunol*. 1995;7(1):41-47. doi:10.1016/0952-7915(95)80027-1
46. van de Mortel JPM, Budding K, Dijkxhoorn K, et al. The role of complement activation in IgM M-protein-associated neuropathies. *Neurol Neuroimmunol Neuroinflamm*. 2025;12(1):e200339. doi:10.1212/NXI.0000000000200339

47. Idusogie EE, Wong PY, Presta LG, et al. Engineered antibodies with increased activity to recruit complement. *J Immunol*. 2001;166(4):2571-2575. doi:10.4049/jimmunol.166.4.2571
48. Haslund-Gourley B, Woloszcuk K, Hou J, et al. IgM N-glycosylation correlates with COVID-19 severity and rate of complement deposition. *Nat Commun*. 2024;Jan 9(15(1)):404. doi:10.1038/s41467-44211-0
49. Gironi M, Saresella M, Marventano I, et al. Distinct cytokine patterns associated with different forms of chronic dysimmune neuropathy. *Muscle Nerve*. 2010;42(6):864-870. doi:10.1002/mus.21786
50. Kiefer R, Kieseier BC, Stoll G, Hartung HP. The role of macrophages in immune-mediated damage to the peripheral nervous system. *Prog Neurobiol*. 2001;64(2):109-127. doi:10.1016/s0301-0082(00)00060-5