Complement Activation Is a Prominent Feature of MOGAD

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Myelin oligodendrocyte glycoprotein (MOG)-antibody (Ab)–associated diseases (MOGADs) account for a substantial proportion of pediatric and adult patients who present with acquired demyelinating disorders. Its pathogenesis and optimal therapy are incompletely understood. We profiled systemic complement activation in adult and pediatric patients with MOGAD compared with patients with relapse-onset multiple sclerosis, patients with neuromyelitis optica spectrum disorder, and pediatric control and adult healthy donors. Proteins indicative of systemic classical and alternative complement activation were substantially increased in patients with MOGAD compared to control groups. Elevated levels were detected in both adult and pediatric cases and across all clinical syndromes. Complement inhibition should be explored for its therapeutic merit in patients with MOGAD.

Methods

Patients with MOGAD were recruited from tertiary neurology clinics in Australia, New Zealand, Germany, and Austria (demographic and clinical characteristics are provided in the Table). Presence of MOG-Ab was confirmed using cell-based assays in Sydney, Australia, and Innsbruck, Austria, as recently described. Patients with relapse-onset multiple sclerosis (RMS; n = 34; age range [years] = 20–60; mean = 36.2; median = 32.5), patients with AQP4-IgG seropositive NMOSD as defined by the 2015 International Panel for NMO Diagnosis (IPND) criteria (n = 13; age range [years] = 22–81; mean = 56.2; median = 60) and healthy donors (HDs; n = 38; age range [years] = 21–65; mean = 38.8; median = 40) were recruited at the University Hospital Münster, Germany. Pediatric controls (PCs; n = 16; age range [years] = 4–17; mean = 10.7; median = 11.5) were additionally recruited at the same site which also recruited patients with MOGAD (Children’s Hospital in Datteln, Germany). Human research ethics approvals were granted by the individual ethics committees for the participating hospitals. Informed consent was obtained from patients and controls. In patients with relapses, blood samples were taken within the first 2 weeks after the onset of the clinical episode. Upon venipuncture, samples from patients and...
# TABLE. Demographic and Clinical Characteristics of Individuals Included

| MOGAD Adults | MOGAD Children | PC | RMS | NMOSD | HD |
|--------------|----------------|----|-----|-------|----|
| N            | 38             | 71 | 16  | 34    | 13 | 38 |
| Female       | 29             | 41 | 12  | 25    | 10 | 29 |
| Age (mean, median, SD) | 41.2, 38, 14.4 | 8.4, 9, 4.4 | 10.7, 11.5, 4.5 | 36.2, 32.5, 11.7 | 56.2, 60, 19.9 | 38.8, 40, 15.6 |
| Age range (y) | 18–70          | 1–17 | 4–17 | 20–60 | 22–81 | 21–65 |
| Immunotherapy | Steroids (n = 2) | Treatment naïve (n = 15) | No immunomodulatory, antibiotic or anticonvulsant therapy | Treatment naïve (n = 34) | Rituximab (n = 9) | NA |
|               | No information (n = 36) | Steroids (n = 6) | SCIg (n = 1) | Steroids+IVIg (n = 1) | Cyclophosphamide (n = 1) | Treatment naïve (n = 3) |
| Status at time of blood draw | Onset (n = 16) | Relapse (n = 6) | Remission (n = 7) | No information (n = 9) | NA | Relapse (n = 14) | Remission (n = 20) | Remission (n = 10) | NA |
| Clinical diagnoses | Unilateral optic neuritis (n = 14), bilateral optic neuritis (n = 17), longitudinal extensive transverse myelitis (n = 7) | Unilateral optic neuritis (n = 22), bilateral optic neuritis (n = 2), longitudinal extensive transverse myelitis (n = 7), acute disseminated encephalomyelitis (n = 40) | Syncope (n = 2), RMS (n = 1), first epileptic seizure (n = 1), opsoclonus myoclonus syndrome (n = 1), complex regional pain syndrome (n = 1), headache (n = 1), optic disc drusen (n = 1), transient visual impairment (n = 1), peripheral facial palsy (n = 1), muscle weakness (n = 1), ophthalmoplegia (n = 1), toe walking (n = 1), psychosis (n = 1), global developmental delay (n = 1), radiologically isolated syndrome (n = 1) | RMS (n = 34) | NMOSD (n = 13) | NA |

HD = healthy donors; MOGAD = MOG-antibody associated disease; NA = not applicable; NMOSD = neuromyelitis optica spectrum disorder; PC = pediatric controls; RMS = relapse-onset multiple sclerosis.
from control individuals were held at room temperature for 30 minutes to allow for clot retraction then centrifugation at 4°C was performed and serum specimens were subsequently frozen down at −70°C. For quantification of complement protein levels, samples were thawed on ice and immediately processed. All samples were handled in
the same manner and all underwent the same number of freeze/thaw cycles. A multiplex enzyme-linked immunosorbent assay (ELISA) based on chemiluminescence was used according to the manufacturer’s recommendations (Quidel, San Diego, CA, USA; cat. number: A900) to systematically profile complement proteins in serum samples. Serum specimens from healthy individuals that had been stored at −70°C and thawed on ice once or twice or freshly processed without freezing showed similar levels of activated complement proteins (data not shown). All samples were run in duplicates. Each plate contained samples from different clinical cohorts to minimize inter-plate variations. Control samples provided by the manufacturer were included on each plate to assure for plate-to-plate consistency. Data were obtained with Imager L from Quansys, using Q-View Software version 3.11 for analysis. The Mann–Whitney test was performed to compare levels of complement proteins between clinical cohorts. The conservative Bonferroni correction method was applied to adjust for multiple testing. GraphPad-Prism version 9.0 was used for statistical analyses.

Results

Systemic complement activation was evaluated in 109 patients with MOGAD compared with 34 patients with RMS, 16 PCs, 13 patients with NMOSD, and 38 HDs (see the Table). All patients with MOGAD were seropositive for MOG-IgG as quantified by cell-based assays. Pediatric patients (n = 71) presented with acute disseminated encephalomyelitis (ADEM; n = 40), unilateral optic neuritis (ON; n = 22), bilateral ON (n = 2),
and transverse myelitis (TM; n = 7). Adult patients (n = 38) developed unilateral ON (n = 14), bilateral ON (n = 17), or TM (n = 7). Disease course was monophasic in the majority of patients, whereas 32% of pediatric and 39% of adult patients experienced relapses (Fig 1A).

Proteins indicative of activation of the classical complement pathway (CP) and the alternative pathway (AP) were substantially increased in patients with MOGAD compared to control patients with RMS, PC, and HD. With the exception of C3c, levels in patients with MOGAD were also higher if compared to patients with NMOSD (Fig 1B–F). In line with the finding that activation levels of the complement CP and AP increase with age,7 pediatric patients with MOGAD showed similar (C3a, SC5b9, and Factor H) or slightly lower (Ba, Bb, and C5a) serum levels of complement proteins as compared to adult patients (Fig 2A–F). Patients with monophasic disease did not differ from patients with relapsing disease in levels of any of the proteins studied (see Fig 2A–F). Complement levels in patients classified as clinically active were not significantly different from those in patients in remission at the time of blood sampling (data not shown). Increased serum concentrations of activated complement proteins were observed across all of the aforementioned clinical MOGAD phenotypes (Fig 3A–F).

**Discussion**

To our knowledge, this is the first study reporting systemic complement activation in patients with MOGAD. Substantially increased complement activation was detectable in both pediatric and adult patients with MOGAD compared with patients with RMS, NMOSD, pediatric control patients, and healthy volunteers. These data indicate that activated complement proteins may contribute to central nervous system (CNS) tissue damage and suggest that inhibition of complement activation should be explored for its therapeutic merit in patients with MOGAD.

Levels of activation products were unchanged in multiple sclerosis (MS) and NMOSD when compared to HDs and were substantially lower compared to MOGAD. Previous studies reported elevated, unchanged, or decreased plasma or serum levels of individuals or a combination of a few complement proteins in patients with MS and NMOSD when compared to HD.8–12 Complement deposition is a characteristic feature of MS lesions only in a subset of patients (MS type II pathology),13 and disease heterogeneity might have contributed to inconsistencies in aforementioned studies. The finding that levels of activated complement components are not increased in NMOSD as compared to MS might partly be explained
by ongoing immunotherapy in the patients recruited and should be an incentive for profiling complement activation in larger treatment-naïve cohorts. A recent smaller scope study reported serum complement C4 levels to be higher in 15 patients with MOGAD as compared with 16 patients with NMO.14 However, most of the patients in the aforementioned study received immunosuppressive therapy and the assay used did not specifically quantify the activation of the complement proteins.

Our findings in patients with MOGAD support recent histopathological investigations in biopsy cohorts. Höftberger et al demonstrated profound deposition of the activated terminal complement complex (C9neo antigen) across all active white matter lesions in all patients investigated indicating that complement activation is a characteristic feature of CNS lesions in MOGAD.15 Takai et al primarily studied the cellular composition of MOGAD lesions in biopsies taken from Japanese patients but also described C9neo antigen deposition, although at lower frequencies as in the aforementioned study. Ethnic differences might account for less abundant lesion-associated complement deposition in Japanese patients as the clinical presentation and prognosis appears also different as compared to White patients.17

Most native MOG-Abs are complement-fixing IgG1 isotypes.18 Pathogenic mechanisms of MOG-IgG might include functions mediated by the antibody’s antigen-binding domain, such as cytoskeleton alterations in oligodendroglial cells,19 but the demyelinating potential of MOG-IgG in vivo upon adoptive transfer into susceptible rodents is related to their ability to activate complement.20,21 The complement signature in patients with MOGAD identified in this study is compatible with IgG-mediated activation of the classical pathway and supports experimental and pathological data highlighting a central role for complement activation in MOG-Ab induced demyelination. The AP of complement is continuously activated and can act as an amplification loop for all 3 pathways, as it is initiated by the binding of C3b.2,22 Initial Ab-mediated activation of the classical complement cascade can, therefore, amplify AP activation. Activation of the AP can further be increased through recognition of apoptotic and necrotic cell surfaces during inflammatory tissue damage.2,22 Currently, there are no established parameters that allow a reliable risk evaluation for the occurrence of further relapses or disability in patients with MOGAD. In our cross-sectional analysis, levels of complement activation products in patients with monophasic versus relapsing diseases or in patients with clinical active disease versus remission did not differ, suggesting that immune and CNS-intrinsic mechanisms contributing to MOGAD are complex and probably not restricted to complement activation. Longitudinal measurements with integrated analysis of clinical parameters are needed to better evaluate the predictive value of individual complement analytics for relapses, disability outcomes, and in guiding treatment decisions. Furthermore, information on immunotherapy at the time of blood draw was not available for all patients with MOGAD. However, we consider it unlikely that immunosuppressive treatment would enhance systemic complement activation.

Markers indicative of systemic IgG-mediated complement activation are prominently increased in children and adults with MOG-Ab associated acquired demyelinating syndromes. These data suggest that patients with MOGAD might benefit from pharmacologic complement inhibition.

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Author Contributions
C.W.K. and J.D.L. contributed to the conception and design of the study. C.W.K., J.A.L., E.M.W., S.R., C.C.G., L.K., M.R., R.C.D., H.W., K.R., and F.B. contributed to the acquisition and analysis of data. C.W.K. and J.D.L. contributed to the drafting of the text and preparing the figures.

Potential Conflicts of Interest
Nothing to report.

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