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REVIEW ARTICLE

Detection of MOG-IgG by cell-based assay: moving from discovery to clinical practice

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Abstract
Myelin oligodendrocyte glycoprotein (MOG) is a unique CNS-specific mammalian protein that is expressed on the surface of compact myelin and oligodendrocyte cell bodies. MOG is an accessible target for autoantibodies, associated with immune-mediated demyelination in the central nervous system. The identification of MOG reactive immunoglobulin G antibodies (MOG-IgG) helps to distinguish a subgroup of patients from multiple sclerosis and other CNS disorders, reducing the risk of clinical misdiagnosis. The development of the cell-based assays (CBA) improved the detection of clinically meaningful MOG-IgG binding to conformational MOG expressed in the cell membrane surface. In this review, we describe factors that impact on the results of CBA, such as MOG conformation, protein glycosylation, addition of fluorescent tags, serum dilution, secondary antibodies, and data interpretation.

Keywords Myelin oligodendrocyte glycoprotein · Autoantibodies · CBA · Neuromyelitis optica spectrum disorder · Acute disseminating encephalomyelitis · Multiple sclerosis

Introduction

Myelin oligodendrocyte glycoprotein (MOG) is a protein expressed on the surface of oligodendrocytes [1]. It is found throughout the central nervous system (CNS) in the brain, optic nerves, and spinal cord [2]. MOG has been identified as a target of immunoglobulin G antibodies (MOG-IgG), which has been associated with a subgroup of immune-mediated CNS demyelinating diseases [3].

MOG antibodies were initially reported in association with multiple sclerosis (MS). However, these antibodies were detected by ELISA or western blot (WB). These tests also detected antibodies in healthy individuals and infectious control populations suggesting a lack of clinical utility in detecting MOG antibodies using these methods [4]. Subsequently, the role of conformational sensitive MOG-IgG began to be investigated in other inflammatory demyelinating diseases of the CNS [5]. Even detecting antibodies against native protein demonstrates MOG-IgG in healthy individuals. However, when serum is diluted much more than in other tests, a clinical association appears. MOG-IgG was found in patients with any combination of optic neuritis, cortical encephalitis and/or myelitis, acute disseminated encephalomyelitis (ADEM), and aquaporin-4 antibody negative neuromyelitis optica spectrum disorder (NMOSD) [2, 6–10]. A new subgroup of demyelinating disorders distinct from MS is being established, but the biology of "MOG-IgG" needs work.

Therefore, the detection of MOG-IgG has utility in clinical practice, but an understanding of the specific MOG assay is important. MOG-IgG detection varies considerably among studies, suggesting low sensitivity or specificity of some methods, leading to inaccurate results [11, 12]. The search for more reliable methods with potential for high-throughput resulted in the development of cell-based assays (CBAs). However, there is a large variation of in-house assay protocols which needs standardization. This review aims to describe advances in the development of MOG-IgG assays and the factors that may influence their accuracy.

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MOG: the antigen

MOG is a quantitatively minor component of myelin that accounts for about 0.05% of total myelin proteins [1, 13]. It is highly homologous among mammals and absent in the brains of birds, reptiles, and lower orders [12, 14]. The MOG gene is located on chromosome 6 in the major histocompatibility complex (MHC) and encoded by 10 exons, alternatively spliced [15, 16] to generate up to 13 human isoforms [17]. These isoforms have identical extracellular immunoglobulin domains, differing only by the cytoplasmic exons that are expressed [12], the last of which determines their α or β classification, as shown in Table 1 [12, 17, 18]. As for composition and molecular weight, there is a range depending on the isoform [15, 16]. Its structure contains an extracellular domain, a transmembrane domain, a cytoplasmic loop, a membrane-associated region, and a cytoplasmic tail [18, 19].

The role of MOG and its isoforms is not clear. Some studies have suggested that MOG may serve as a potential marker of oligodendrocyte maturation, because its expression begins later than that of other proteins in myelination [2, 20]. In addition, given its location and structure, MOG may function as a cell surface receptor or cell adhesion molecule [15, 21]. Another possible function of MOG could be to regulate oligodendrocyte microtubule stability [15, 22]. Moreover, MOG binds to C1q, the first component of the classical pathway of complement [23]. This MOG-C1q binding may mediate the interaction between myelin and the immune system. It has recently been demonstrated that soluble nerve growth factor binds MOG expressed in cell lines which may impact on axon growth modulation, as well as impacting the morphology of the unmyelinated posterolateral tract of the spinal cord [24].

Table 1 MOG isoforms with amino acids and exons

Isoforms	Size (kDA)	Amino acids	Exons
α1	25.1	218	1, 2, 4, 5, 6, 8, 9, 10a
α2	20.5	179	1, 2, 4, 5, 6, 9, 10a
α3	22.2	195	1, 2, 4, 5. 6, 7, 9, 10a
α4	16.3	142	1, 2, 3
β1	25.6	223	1, 2, 4, 5, 6, 8, 9, 10b
β2	20.2	177	1, 2, 4, 5, 6, 10b
β3	22.7	200	1, 2, 4, 5, 6, 7, 9, 10b
β4	16.3	142	1, 2, 3
β5	21.0	184	1, 2, 4, 5, 6, 9, 10b

The composition and molecular weight vary according to the isoform. Isoforms are classified in α and β, which are divided by exons 10a and 10b. The α4 and β4 isoforms are shorter, containing 26 amino acids encoded by exon 3. The other isoforms differ in the inclusion of exons 7 (α3 and β3), 8 (α1 and β1), and 9 (present in all, except α4, β2, β4)

Functional data on MOG are often not reproduced; hence, we are in the early stages in our understanding of what MOG does, and published data needs to be taken with caution.

Role of MOG in demyelination and related diseases

Human studies in the late 1990s identified MOG antibodies in patients with relapsing autoimmune demyelinating diseases [25]. Further work over the following 10 years suggested a potential association between MOG antibodies and the risk of conversion clinically isolated syndromes to MS [26, 27]. However, these studies used ELISA with recombinant linear MOG peptides and WB analysis with denatured MOG. Follow-up studies with larger populations included better controls that demonstrated a lack of clinical utility, indicating that the techniques used in those studies had limitations in identifying clinically meaningful MOG antibodies [4, 5, 28]. In 2007, using a MOG-tetramer radioimmunoassay (RIA), MOG-IgG against native MOG was identified in patients with ADEM [5]. Shortly after, studies using CBA also identified MOG-IgG in patients with ADEM, especially in paediatric patients [28–30]. In 2011, the first report of the presence of MOG-IgG in patients with NMOSD was published [31]. Most patients with NMOSD have aquaporin-4-reactive immunoglobulin G (AQP4-IgG), but MOG-IgG was detected in a proportion of the AQP4-IgG seronegative patients [32, 33].

In addition to ADEM and AQP4-IgG seronegative NMOSD, MOG-IgG antibodies have been recently described in serum samples from patients with an expanding spectrum of non-MS inflammatory CNS demyelinating disorders [6]. Optic neuritis (sometimes bilateral) is the clinical phenotype most commonly associated with MOG-IgG in adults and older children [34, 35]. Myelitis is also a common feature, which can be multiple and mostly longitudinally extensive [36, 37]. More recently, some patients with cortical encephalitis have been described in association with MOG-IgG [8].

A proportion of the MOG-IgG+ patients are monophasic, but there is no definitive biomarkers that can accurately estimate the risk of a relapsing disease [2, 35]. In patients with persistent MOG-IgG, especially with high titres, the risk of relapses seems to be higher than those that became negative during the follow-up [36, 38]. However, one study observed relapses in paediatric patients that may fluctuate MOG-IgG titres over the time, sometimes with negative results. This indicates that we should repeat the MOG-IgG if the patients have new attacks [39]. Moreover, the clinical spectrum associated with MOG-IgG is still expanding. Especially in the paediatric population, MOG-IgG testing should be considered in a broad number of patients presenting with inflammatory CNS disorders [10].

Antibody detection

The irreproducible results of the antibody detection by classical immunoenzymatic assays using different patient populations in different studies suggested a lack of clinical utility [40–42]. The evolution of cell-based assays in neuroimmunology against aquaporin-4 and the target antigens in autoimmune encephalitis led to an understanding that antibody binding to native protein is key to the identification of antibodies that can have a functional impact in vivo. This led researchers to look for alternative methods to detect MOG-IgG that bound native human MOG. O'Connor et al. used a MOG tetramer RIA and identified MOG-IgG in paediatric patients with ADEM but not in children diagnosed with paediatric MS, suggesting that conformational antibodies against MOG may have clinical utility [5]. Meanwhile, CBAs were developed for MOG-IgG detection, with results indicating that this is a reliable technique to identify antibodies that recognize conformational epitopes of MOG in clinical practice, with the caveat that the serum is needed to be diluted more than in similar assays for aquaporin-4 or LGI1, for example [43].

CBAs are based on natively folded MOG expressed on the cell surface serving as a target antibodies [1]. Plasmids, that encode the full-length sequence of human MOG, usually the α1 isoform, are transfected into cells [44]. Liposome-, polyethylenimine- (PEI), or electroporation-based methods can be used for cell transfection [45]. Several eukaryotic cell lines have been used to recombinantly express MOG, such as Chinese hamster ovary (CHO), Jurkat, human LN18 glioblastoma, human rhabdomyosarcoma TE671, and human embryonic kidney 293 (HEK293); the latter is the most widely used cell line (Fig. 1). These cells have sufficient expression of MOG in the cell membrane for antibody detection [12].

Cells expressing MOG are sequentially incubated with diluted patient serum followed by anti-human-specific secondary antibodies after washing. The secondaries are most often conjugated to fluorescein isothiocyanate (FITC), an Alexa Fluor or other fluorophores, and, depending on how they were generated, can recognize all human Ig classes, IgG, or IgG1 only [13]. Subsequently, binding is either quantified by flow cytometry (FACS) or visualized by direct microscopic evaluation of indirect immunofluorescence (IIF) [46]. For MOG-IgG titers, the serum can be progressively diluted up to the loss of the fluorescence signal using microscopy. In FACS assays, antibody titres are evaluated by the ratio or the delta of median fluorescent intensity (MFI) between transfected and untransfected cells [37].

MOG-IgG CBA sensitivity and specificity

Currently, we do not have consensus on the functional impact of MOG-IgG. There is an increasing range of clinical phenotypes associated with MOG-IgG. Until we understand the impact of the antibody in vivo limiting the testing to the most common phenotypes risks missing clinical clues to its function and rare presenting phenotypes. Testing for MOG-IgG by CBA has increased the assay specificity, but the sensitivity and reproducibility still need examination. Factors such as MOG conformation, isoform, cell line used, post-translation modifications, addition of tags, serum dilution, secondary antibody used, and test output will all impact on CBA metrics.

MOG length

MOG has multiple isoforms. The α1 isoform, most commonly used for diagnostic tests, is known as full-length MOG (FL-MOG). However, when a stop codon was introduced after the transmembrane loop after Gly155, resulting in the deletion of 73 intracellular amino acids, a short-length MOG construct was made (SL-MOG) [47]. Antibodies to this artificially truncated form identified only 1/3 of patients captured by the full-length assay [13] and hence were not considered useful for clinical practice. As these isoforms are identical on the extracellular surface, the reason behind the difference in sensitivity of these two proteins is unclear [12]. There are at least 12 other human isoforms described at the mRNA level in multiple databases, seven of which have been detected at the protein level using antibodies [17]. An understanding of the distribution of these proteins and the binding capacity of antibodies to these isoforms are lacking.

Fluorescent tags

Some authors have performed CBA using MOG covalently linked with a green fluorescent protein (GFP), as shown in Fig. 1. GFP is a 238 amino acid protein with molecular weight of 27–30 kDa that is used as a fluorescent tag to localize proteins in living cells [48]. GFP is usually expressed fused to the target protein at the N or C termini but can be inserted anywhere [49]. However, covalent attachment of GFP, or similar molecules, to MOG may impact on its folding, surface expression, or quaternary structure on the cell membrane. In addition, high expression levels of fluorescent proteins can be cytotoxic to the cells in which they are expressed leading to apoptosis [50]. Liu et al., in a study conducted in 1999, revealed that GFP-expressing cells contracted and rounded-up, or died [51]. Conversely, others have shown that the fusion of GFP to MOG does not alter its antibody-binding capability when compared directly with its GFP untagged native counterpart suggesting that the cells are healthy enough to function as an assay substrate [52].

Another fluorescent marker used in mammalian cells is the red fluorescent proteins DsRed from the sea anemone (*Discosoma* sp.) [53]. This red marker is often encoded in

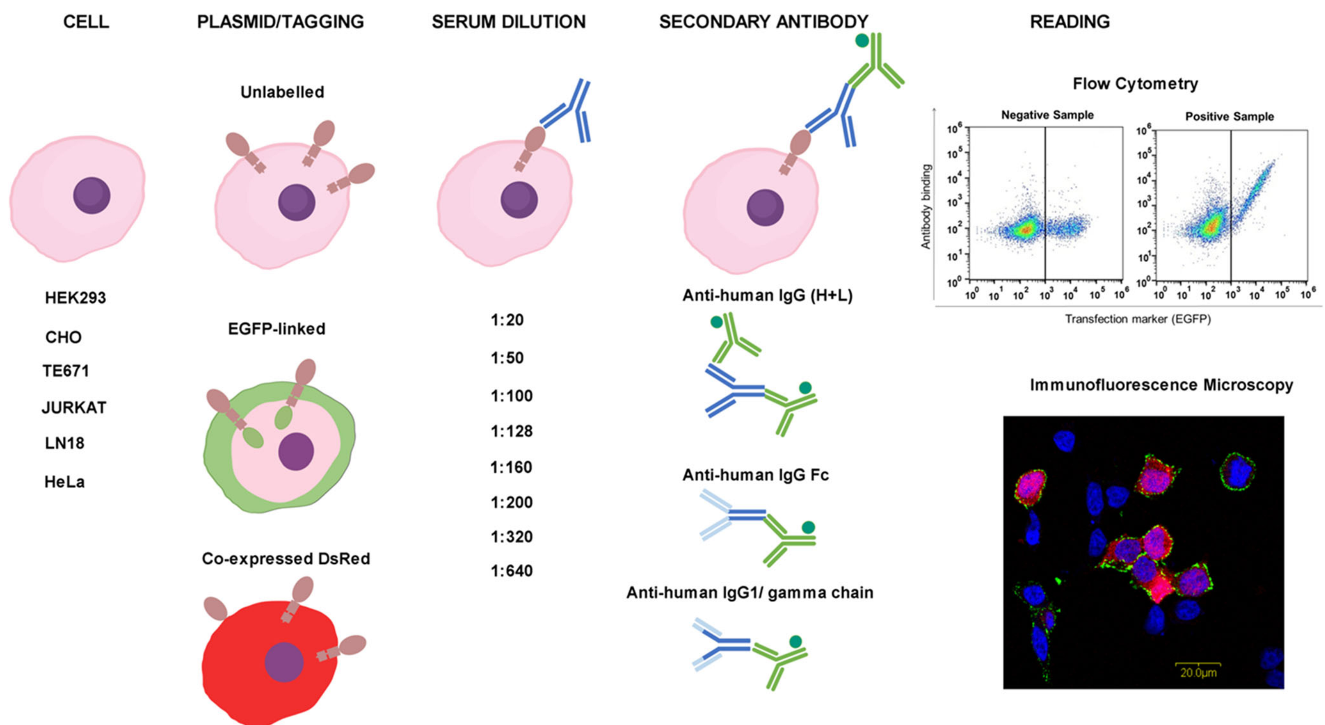

Fig. 1 Overview of MOG-IgG assays. Cell-based assays (CBA) are performed based on natively folded MOG expressed on the cell surface serving as a target for the action of antibodies. The plasmid can be unlabelled, MOG can be covalently attached to eGFP (EGFP-linked), or MOG can be encoded in the same plasmid as DsRed (co-expressing DsRed). Transfected MOG-cells are exposed to diluted human serum and then labelled with secondary antibodies. The anti-human IgG (H + L) binds to all classes of antibodies, the anti-human IgG Fc-specific binds to the Fc portion of all IgG subclasses, and the anti-human IgG1/gamma chain binds only to IgG1 subclass. The analysis is performed by flow cytometry or indirect immunofluorescence microscopy

the same plasmid as MOG, but not covalently linked to it. The DsRed is encoded after an internal ribosome entry site which means it ends up expressed in cell cytosol, lighting up cells while MOG remains native and is targeted to the membrane. However, proteins that are expressed after an IRES are expressed less well than those encoded at the start of the mRNA; hence, you can get cells expressing MOG with very low levels or absence of DsRed.

Serum dilution

Dilution is another factor that varies widely among studies (Fig. 1). MOG-IgG antibodies are prevalent; hence, a serum cut-off of 1:160 is used at most centres. This means that healthy people will have MOG-specific IgG in their blood but only detectable at low serum dilutions, particularly children, but no clinical association with MOG-IgG at this level has been established. Most other antigens are screened at a serum dilution of 1:20 [12]. The higher the serum concentration used in CBA-IIF, the greater the likelihood that background staining appears which can make the subjective, semi-quantitative scoring by microscopy difficult [54]. The experience of the individual running the test and keeping the cells healthy can be key. A low serum dilution may increase the background fluorescence in the CBA-FACS, caused by non-specific IgG binding to cell membrane [12, 54]. However, this background can be reduced using higher dilutions [37, 55], but there is always a risk of reducing sensibility of the MOG-IgG assay.

Secondary antibody

A high rate of clinically irrelevant positive results was observed in the first generation of MOG-IgG assays, and increasing serum dilution has reduced this effect. A second issue was the use of anti-human IgG (H + L) secondary antibodies which can bind to the common light chain of any antibody class. A few cases with IgM antibodies were identified, and they did not have typical clinical features associated with MOG-IgG [56]. Thus, knowing that the majority of MOG-IgG-positive cases are IgG1 subtype, the use of an anti-human IgG1 or anti-human IgG-Fc cross-absorbed secondary antibody provides clearer results [13, 57]. We have a previous study demonstrating that few controls had some positivity at 1:20 dilution using secondary IgG (H + L), but they were negative when using secondary antibody for IgG1 [47]. For clinical testing, secondary anti-human IgG-Fc seems to be more specific than IgG (H + L) [58]. Therefore, the use of highly specific secondary antibodies to IgG is recommended

to avoid cross-reactivity to other immunoglobulin classes and reduce the risk of false-positive results [12, 47].

Analysis by flow cytometry and immunofluorescence microscopy

Antibodies in patient sera binding to transfected cells can be analysed by microscopy or flow cytometry (Fig. 1). CBA-IIF is a subjective technique that requires an experienced observer to recognize binding patterns by microscopy in order to score the binding correctly. Flow cytometry, on the other hand, is objective and quantitative where each transfected or non-transfected single cell is analysed for expression of MOG by detecting the covalent eGFP tag and human antibody binding in separate channels. The positivity can be evaluated using a ratio of fluorescence intensity or the difference between the mean fluorescence intensity signal between the MOG-transfected and non-transfected cells. This allows for off-target binding directly to the cells to be ruled out. Multiple samples can be run simultaneously, and automatic read-outs are generated [47]. However, there is need for a trained professional to maintain the equipment and running costs are higher.

Few studies compared the two methods for the detection of MOG antibodies with equivalent results when live cells are used, suggesting that these methods are comparable [43, 59, 60]. Flow cytometry tests require further development and standardization. This method should, at worst, be comparable to microscopy for antibody detection and has the advantage of being quantitative and objective [61]. One recent study found a 96% agreement between the two methodologies, discriminating the clearly positive and negative samples [62]. Another study also found a good correlation between the semi-quantitative CBA-IIF titres and the flow cytometry values [60]. However, performance of MOG-IgG assays may vary according to the centre and the assay. The results of a multicentric study with AQP4-IgG assays indicate discrepant performance between laboratories, suggesting that antibody detection using CBA-IIF and flow cytometry requires experienced personnel to avoid low accuracy.

Conclusion

The development of cell-based assays analysed by microscopy or by flow cytometry, using live transfected HEK cells as the assay substrate, allowed the identification of clinically relevant autoantibodies in a subgroup of patients with non-MS inflammatory CNS demyelinating disorders. However, several factors like serum dilution, recombinant constructs with changes in the protein conformation or glycosylation, secondary antibodies, and reading methods may influence the sensitivity and specificity of the CBA. Standardization of these assays is essential for clinical care worldwide.

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Compliance with ethical standards

Conflict of interest The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations ADEM, acute disseminated encephalomyelitis; AQP4, aquaporin-4; CBA, cell-based assay; CHO, Chinese hamster ovary; CNS, central nervous system; eGFP, enhanced green fluorescent protein; ELISA, enzyme-linked immunonosorbent assay; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; FL-MOG, full-length MOG; GFP, green fluorescent protein; HEK293, human embryonic kidney 293; IIF, indirect immunofluorescence; IgG, immunoglobulin G; IgM, immunoglobulin M; LGI1, leucine-rich glioma inactivated 1; MFI, median fluorescent intensity; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; mRNA, messenger ribonucleic acid; NMOSD, neuromyelitis optica spectrum disorder; PEI, polyethylenimine; RIA, radioimmunoassay; SL-MOG, short-length MOG; WB, western blot

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