

【DESCRIPTION】

【Invention Title】

METHOD FOR CELL-BASED ASSAY TO DETECT MOG-IgG IN PATIENTS
WITH CENTRAL NERVOUS SYSTEM INFLAMMATORY DISEASES

【Technical Field】

The present invention relates to a method of detecting myelin oligodendrocyte glycoprotein (MOG)-IgG from a sample, comprising the steps of: (a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; (b) transfecting a cell with the MOG-cloned expression vector; (c) culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line; (d) incubating the selected cell line with a sample expected to contain MOG-IgG; (e) adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and (f) detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate; a method of providing information for prediction or diagnosis of MOG-IgG-related central nervous system inflammatory disease; a composition for diagnosis of MOG-IgG-related central nervous system inflammatory disease; and a method of diagnosing MOG-IgG-related central nervous system inflammatory disease.

【Background Art】

The serostatus of autoantibodies is an important diagnostic criterion in a wide range of neurological diseases, including autoimmune diseases. MOG-IgG-positive patients with various demyelinating phenotypes have been identified due to the development of a cell-based assay (CBA) using myelin oligodendrocyte glycoprotein (MOG) as a substrate. MOG-IgG was present in acute disseminated encephalomyelitis (ADEM), seizures, encephalitis, aquaporin 4 (AQP4)-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), optic neuritis (ON), myelitis, and brainstem encephalitis in children and adults. On the other hand, MOG-IgG was not present in patients with multiple sclerosis (MS), so it was thought to differentiate multiple sclerosis from non-MS central nervous system (CNS) demyelinating disorders. In addition, there are clinical, radiological, pathological and prognostic differences between patients with MS and MOG-IgG-related diseases. According to a recent study, persistent MOG-IgG positivity was associated with clinical relapse despite immunotherapy, whereas conversion to seronegative status did not result in clinical relapse. Furthermore, serological findings and other experimental approaches have suggested that MOG-IgG may be pathogenic. Nevertheless, the spectrum of MOG-IgG-related diseases is still not fully defined. Therefore, the development of sensitive and

highly specific assays to identify specific autoantibodies is important in delineating the full spectrum of MOG-IgG-related diseases by accurately defining the clinical phenotype, disease course, and prognosis.

【Disclosure】

【Technical Problem】

It is an object of the present invention to detect MOG-IgG from a sample such as blood or serum, and furthermore, to reveal the entire spectrum of MOG-IgG-related diseases, by constructing a stable transfected cell line expressing MOG and reacting it with a sample isolated from a patient with central nervous system (CNS) inflammatory disease expected to contain MOG-IgG, and then adding a fluorescence-conjugated secondary antibody that specifically binds to the MOG-IgG, and detecting or analyzing fluorescence therefrom.

It is another object of the present invention to provide a method of providing information for prediction or diagnosis of MOG-IgG-related central nervous system inflammatory disease, wherein the MOG-IgG-related central nervous system inflammatory disease may be AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic neuritis (ON).

It is another object of the present invention to provide a

composition for diagnosis of MOG-IgG-related central nervous system inflammatory disease, produced the steps of: (a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; (b) transfecting a cell with the MOG-cloned expression vector; and (c) culturing the transfected cell in a medium containing a selection drug.

It is another object of the present invention to provide a method for diagnosing MOG-IgG-related central nervous system inflammatory disease.

【Technical Solution】

These will be described in detail as follows. On the other hand, each description and embodiment disclosed in the present application may also be applied to each other description and embodiment. That is, all combinations of the various elements disclosed in this application fall within the scope of this application. In addition, it cannot be seen that the scope of the present application is limited by the specific descriptions described below.

One aspect of the present invention for achieving the above objects provides a method of detecting myelin oligodendrocyte glycoprotein (MOG)-IgG from a sample, comprising the steps of: cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; transfecting a cell with the MOG-cloned

expression vector; culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line; incubating the selected cell line with a sample expected to contain MOG-IgG; adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate.

In the present invention, "myelin oligodendrocyte glycoprotein (MOG)" plays an important role in the myelination of nerves in the central nervous system (CNS), and is a glycoprotein distributed on the surface of the myelin sheath and the plasma membrane of oligodendrocytes. With the development of cell-based assays (CBA) using MOG as substrate, it is known that MOG-immunoglobulin G antibody (IgG), i.e., MOG-IgG, is involved in various central nervous system (CNS) inflammatory diseases. MOG in the present invention is a human full-length MOG (FL-MOG), and the nucleic acid encoding the MOG in the present invention may consist of the base sequence as set forth in SEQ ID NO: 1.

In the present invention, a "stable transfected cell line" refers to a stable cell line expressing the FL-MOG and may be produced by the steps of: cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; transfecting a cell with the MOG-cloned expression vector; and culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line (MOG-HEK293) expressing MOG. Specifically,

the fluorescent protein may be EGFP, GFP, YFP, DsRed2, Tdtomato, or mCherry, and the expression vector may be a pIRES2-EGFP or a pIRES-DsRed2 vector. More specifically, the MOG-cloned expression vector in the present invention may be cloned with the human full-length MOG (FL-MOG) encoded by the base sequence as set forth in SEQ ID NO: 1 in the pIRES2-EGFP vector. In addition, the cells to be transfected may be mammalian cells, that is, human embryonic kidney (HEK) 293 cells, HeLa cells, Chinese hamster ovary (CHO) cells, or NIH3T3 cells, and the selection drug may be, but is not particularly limited to, hygromycin, puromycin, blasticidin, or G418 (geneticin). For example, in one embodiment of the present invention, the human full-length MOG (FL-MOG) cDNA was isolated from the FL-MOG/pIRES2-DsRed2 plasmid, cloned into the XhoI and SmaI sites of the pIRES2-EGFP vector, and transfected into HEK293 cells using Lipofectamine 2000 reagent, and then a stable transfected cell line was selected through a medium containing G418 as a selection drug, wherein the process of extracting or isolating the FL-MOG cDNA and cloning it into an expression vector may be performed by a person skilled in the art by appropriately selecting a method known in the art.

On the other hand, the stable cell line expressing FL-MOG is for detecting MOG-IgG in the sample and further for diagnosing MOG-IgG-related central nervous system inflammatory disease, and the "sample" in the present invention may refer to spinal fluid, blood or serum, more preferably blood or serum, isolated from a

patient with MOG-IgG-related central nervous system inflammatory disease or from a patient expected to contain MOG-IgG. The sample may be used by a person skilled in the art by diluting it at an appropriate ratio as needed.

In the present invention, the "MOG-IgG-related central nervous system inflammatory disease" may appear in MOG-IgG-positive patients with various demyelinating phenotypes, and specifically, may refer to, but is not particularly limited to, AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic neuritis (ON).

In the present invention, the MOG-IgG and secondary antibody conjugate is formed by incubating the stable cell line expressing FL-MOG with the isolated blood or serum, and then adding a fluorescence-conjugated secondary antibody to the incubated sample. In the present invention, the "fluorescence-bound secondary antibody" is for a cell-based assay (CBA) for MOG or MOG-IgG, and the secondary antibody may in particular be anti-human IgG (H+L). Specifically, in one embodiment of the present invention, it was confirmed that when using fluorescence-conjugated IgG (H+L) as a secondary antibody that specifically binds to MOG-IgG, there is a strong positive correlation between CBA-IF and CBA-FACS results, and a high matching rate of about 98% is shown even compared with that of the existing IgG1 (Oxford) CBA-IF, and in particular, it was confirmed that when using fluorescence-conjugated IgG (H+L),

there is advantageous in that no cross reaction with secondary antibody IgM is shown, and thus a clear fluorescence signal having higher specificity may be obtained.

The step of "detecting or analyzing fluorescence" in the present invention may be performing, but is not particularly limited to, an immunofluorescence assay (IF) or a flow cytometry assay (FACS). In one embodiment of the present invention, CBA-IF (NCC-CBA) was performed using three different secondary antibodies, i.e., Alexa Fluor-594-conjugated goat anti-human IgG (H+L), Alexa Fluor-594-conjugated goat anti-human IgG1-Fc and Alexa Fluor-594-conjugated goat anti-human IgM, against MOG-HEK293 cells, and the specificity and sensitivity to MOG of three different secondary antibodies (anti-human IgG (H+L), IgG1-Fc and IgM) were measured via CBA-FACS.

Another aspect of the present invention for achieving the above objects provides a method of providing information for prediction or diagnosis of MOG-IgG-related central nervous system inflammatory disease, comprising the steps of: cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; transfecting a cell with the MOG-cloned expression vector; culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line; incubating the selected cell line with a sample expected to contain MOG-IgG; adding a fluorescence-conjugated secondary antibody to the

incubated sample to form a MOG-IgG and secondary antibody conjugate; and detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate. The term "MOG-IgG-related central nervous system inflammatory disease" is the same as described above.

Since MOG-IgG-related central nervous system inflammatory disease, including neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic neuritis (ON), may occur in MOG-IgG-positive patients with various demyelinating phenotypes, it is possible to provide information for prediction or diagnosis of MOG-IgG-related central nervous system inflammatory disease by checking the presence, level, and the like of MOG-IgG in the sample through the above method.

Specifically, when the stable cell line expressing FL-MOG and a sample expected to contain MOG-IgG are incubated together and a secondary antibody that specifically binds to MOG-IgG is added, MOG-IgG is released from the sample, red fluorescence is observed around the cell membrane due to FL-MOG and MOG-IgG binding when MOG-IgG is present in the sample, the presence or absence of MOG-IgG in the sample may be confirmed through the fact that red fluorescence is not observed when MOG-IgG is not present in the sample, and furthermore, it may be used to analyze the MOG-IgG-expressed level, clinical phenotype, disease course, and prognosis in a patient group with MOG-IgG-related central nervous system inflammatory disease, and to classify the patient group.

Another aspect of the present invention for achieving the above objects provides a composition for diagnosis of MOG-IgG-related central nervous system inflammatory disease, produced the steps of: cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; transfecting a cell with the MOG-cloned expression vector; and culturing the transfected cell in a medium containing a selection drug. The term "MOG-IgG-related central nervous system inflammatory disease" is the same as described above.

Another aspect of the present invention for achieving the above objects provides a method of diagnosing MOG-IgG-related central nervous system inflammatory disease, comprising the steps of: cloning a nucleic acid encoding MOG into a fluorescent protein expression vector; transfecting a cell with the MOG-cloned expression vector; culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line; incubating the selected cell line with a sample expected to contain MOG-IgG; adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate. The term "MOG-IgG-related central nervous system inflammatory disease" is the same as described above.

【Advantageous Effects】

According to the present invention, when fluorescent-conjugated IgG (H+L) was used as a secondary antibody that specifically binds to MOG-IgG, it was confirmed that there was a strong positive correlation between CBA-IF and CBA-FACS results, and a high concordance rate of about 98% was shown even compared to that of conventional IgG1 (Oxford) CBA-IF, and in particular, the method of the present invention has the advantages that by producing a stable cell line expressing MOG, repeated transfection is not required and cross-reaction with the secondary antibody IgM does not occur, so a high specificity and a clearer fluorescence signal may be obtained.

【Description of Drawings】

Figures 1A and 1B show the results of confirming transfection by Western blot and flow cytometry for hMOG-HEK293 cell line transfected with FL-MOG, respectively, and Figures 1C to 1E show the results of observing fluorescence signals when treated with IgG (H+L), IgG1-Fc and IgM as secondary antibodies for control (HC), MOG-IgG-negative and positive patient sera, respectively.

Figure 2A shows the results of performing CBA-FACS using IgG (H+L), IgG1-Fc and IgM as secondary antibodies for the sera of patients with CNS inflammatory diseases and controls (HC), respectively, and Figure 2B shows the results of calculating the

ROC curves and the area under the curve (AUC) for the MFI ratio for IgG (H+L) and IgG1-Fc, respectively.

Figure 3 shows the calculation of the optimal cut-off in the ROC curve for patients with various CNS inflammatory diseases, and the distribution of the MFI ratio for each disease.

Figure 4 shows the results of confirming the correlation between CBA-FACS and the IF scores and CBA-IF scores from MFI ratios in 40 MOG-IgG-seropositive patients.

Figures 5A and 5B show the results of observing fluorescence signals in FL-MOG-transfected FL-MOG + HEK293 cell lines and EV-transfected HEK293 cell lines, and the results of CBA-FACS using IgG (H+L), IgG1-Fc and IgM as secondary antibodies, respectively.

【Mode for Carrying out the Invention】

Hereinafter, the present invention will be described in more detail through the following examples. However, these examples are only for illustrating the present invention, and the scope of the present invention is not limited to these examples only.

Example 1: Experimental method

1-1: Patient group

Serum from 355 patients diagnosed with CNS inflammatory disease at the Korean National Cancer Center (NCC) and 25 healthy

people as a control group (HC) were used. The demographics of the 355 patient groups are shown in Table 1, and their serostatus was assessed by in-house CBA. The patient group included 333 randomly selected patients with CNS inflammatory disease treated at the NCC and 22 patients previously identified as MOG-IgG-positive by IgG1 Oxford CBA (Oxford-CBA).

【Table 1】

Diagnosis at last follow-up	Number of patients	Male: Female	Average age at sampling	Mean prevalence period at sampling	Number of MOG-IgG-positive patients
AQP4-positive NMOSD	65	11:54	37 (13-67)	43 (14-73)	0/65 (0%)
AQP4-negative NMOSD	37	9:28	41 (12-72)	29 (0-351)	4/37 (10.8%)
Multiple sclerosis	111	34:77	32 (16-65)	24 (0-286)	0/111 (0%)
Single or recurrent myelitis	52	32:20	40 (14-74)	18 (0-196)	7/52 (13.5%)
Single or recurrent optic neuritis	16	6:10	34 (15-63)	2 (0-87)	6/16 (37.5%)
Other demyelinating disorders, including neoplastic demyelination or ADEM-like symptoms	55	23:32	34 (7-61)	7 (0-325)	24/55 (43.6%)
Other neurological disorders	19	11:8	49 (21-68)	6 (0-212)	0/19 (0%)

The specificity and sensitivity of three different secondary antibodies (anti-human IgG (H+L), IgG1-Fc and IgM) in 105 patients

were evaluated by flow cytometry, and the specificity and sensitivity of the secondary antibody was confirmed by CBA-IF in 10 patients. The match between NCC CBA-IF (NCC-CBA) and Oxford-CBA-IF was evaluated in 125 patients (including 22 MOG-IgG-seropositive patients). Serum collected from all participants was stored at -80°C, and the diagnosis of NMOSD and MS was based on the 2015 International NMOSD Diagnostic Panel criteria and the 2010 McDonald's criteria, respectively.

1-2: Production of MOG-HEK 293 cells

Human full-length MOG (FL-MOG) cDNA was isolated from the FL-MOG/pIRES2-DsRed2 plasmid and cloned into the XhoI and SmaI sites of the pIRES2-EGFP vector (Clontech, Mountain View, CA, USA). All sequences were confirmed by automated sequencing. Thereafter, the cloned FL-MOG plasmid or empty vector plasmid was transfected into human embryonic kidney 293 (HEK293) cells (ATCC, Manassas, VA, USA) using Lipofectamine 2000 reagent (Invitrogen, Waltham, MA, USA). After 48 hours, the cells were divided into the media containing 2 mg/mL G418 (Invitrogen), and after 2 weeks, G418-resistant cells were isolated and further cloned by limited dilution. Each stable transfectant clone was screened for green fluorescent protein (GFP) expression using flow cytometry. FL-MOG protein expression in GFP-positive cells was confirmed by Western blot using an anti-MOG antibody (Santacruz, Dallas, TEXAS, USA).

**1-3: MOG-IgG cell-based indirect immunofluorescence assay
(CBA-IF)**

CBA-IF was performed using three different secondary antibodies, i.e., Alexa Fluor-594-conjugated goat anti-human IgG (H+L) (diluted 1:2000 with PBS), Alexa Fluor-594-conjugated goat anti-human IgG (FC γ fragment-specific; diluted 1:750 with PBS), Alexa Fluor-594-conjugated goat anti-human IgM (diluted 1:750 with PBS), against MOG-HEK293 cells. Empty vector (EV)-stably transfected HEK293 cells were used as a control group to exclude non-specific background staining. Each experiment was performed in duplicate, and seropositivity was determined by two investigators unaware of the patient's clinical and experimental information and each other's results. The intensity of surface immunofluorescence was scored as shown in Table 2 below, and the final score is the median of readings from two or three investigators. An IF score of 1 or higher was considered positive and the score of 0.5 was considered borderline.

【Table 2】

IF score	Description
0	There is no binding around the cell membrane, or the staining does not surround the cell membrane in a ring shape.
0.5	There is a weak binding around the cell membrane, but the staining does not surround the entire membrane in ~50% of prickle cells.
1	There is a clear and weak binding, and the staining surrounds the entire membrane in a ring shape in >50% of prickle cells.

2	There is a clear and moderately bright binding, and the staining surrounds the entire membrane in >75% of prickly cells.
3	There is a clear and bright binding, and similar to the brightness of the positive control in >90% of prickly cells.
4	There is very bright binding, and stronger than the positive control in >90% of prickly cells.

1-4: Cell-based flow cytometry (CBA-FACS)

HEK293 cells were harvested and washed in staining buffer (2 mM EDTA-1X PBS supplemented with 0.5% bovine serum albumin). 2×10^5 cells were cultured with patient serum (diluted 1:40 with staining buffer; 200 μ l) for 30 min in 96-well U-bottom plates (Thermo Fisher Scientific; MA, USA) on ice. Cells were then washed twice with PBS and cultured with allophycocyanin-conjugated goat anti-human IgG (H+L) (Jackson Immunology; PA, USA; diluted 1:2000 with PBS), phycoerythrin-conjugated mouse anti-human IgG1 Fc (SouthernBiotech; Birmingham, USA; diluted 1:1000 with PBS) or blue-violet 421-conjugated mouse anti-human IgM (BD Bioscience; CA, USA; diluted 1:50 with PBS) on ice for 30 min. The cells were washed and resuspended in 400 μ l of cold PBS, and then viability dye 7-AAD (BD Biosciences; CA, USA; 5 μ l per sample) was added to the cells to exclude dead cells before harvesting. A total of 10,000 cells were harvested from FACSVerse and data were analyzed using Flow Jo software (TreeStar; Ashland, OR, USA). Binding was expressed as mean fluorescence intensity (MFI), and the MFI ratio

was determined by dividing the MFI of MOG-transfected HEK293 cells by the MFI of EV-transfected HEK293 cells.

CBA-IF was used as a reference standard, and receiver-operating characteristic (ROC) curve analysis was used to determine the usefulness of the MFI ratio for determining MOG-IgG seropositivity. The optimal cut-off of the highest specificity and sensitivity was determined through ROC curve analysis. Specificity was calculated as $[(\text{Number of MOG-IgG negative among patients with AQP4-IgG positive NMOSD, MS and other neurological diseases (OND)}) / (\text{Total number of patients with AQP4-IgG positive NMOSD, MS and OND})] \times 100$.

Example 2: Experimental results

2-1: In-house MOG-IgG CBA-IF

FL-MOG-stably transfected HEK293 cells were used to construct in-house CBA-IF, successful transfection was confirmed by Western blot and flow cytometry, and fluorescence levels were measured by fluorescence microscopy (Figures 1A and 1B).

Previous studies had concerns about the low specificity in the use of anti-human IgG (H+L) secondary antibodies to detect MOG-IgG, especially due to the cross-reactivity of IgG and IgM antibodies in MS patient samples. On the other hand, IgG Fc or IgM secondary antibodies were used for CBA-IF with a limited number of samples (n = 10; 1 AQP4-IgG-negative NMOSD, 2 single or recurrent myelitis, 4 other demyelinating diseases (ODD) including

neoplastic demyelination or ADEM-like symptoms, and 3 healthy control).

As a result, it was confirmed that there was no difference in fluorescence between IgG (H+L) and IgG Fc, and in particular, no fluorescence signal was seen when an IgM secondary antibody was used (Figures 1C to 1E). Specifically, green fluorescence was observed in the cytosol of MOG-transfected cells, and red fluorescence was observed around the cell membrane by conjugation of MOG-IgG to FL-MOG when patient serum containing MOG-IgG was added (Figure 1E). On the other hand, it was confirmed that red fluorescence was not observed when the patient serum did not contain MOG-IgG (Figures 1C and 1D).

2-2: MOG-IgG serostatus

Serostatus of 25 HC and 355 patients (65 AQP4-IgG-positive NMOSD, 37 AQP4-IgG-negative NMOSD, 111 MS, 52 single or recurrent myelitis, 16 single or recurrent optic neuritis (ON), 55 ODD, 7 OND) from the CNS inflammatory disease population was evaluated. Characteristics and MOG-IgG seropositivity of 355 patients are shown in Table 1. 41/355 (11.5%) of the total 355 patients were seropositive, wherein 4/37 (10.8%) patients with AQP4-IgG-negative NMOSD, 7/52 (13.5%) patients with single or recurrent myelitis, 6/16 (37.5%) patients with single or recurrent ON and 24/55 (43.6%) patients with ODD were confirmed to have MOG-IgG in their serum. In addition, none of the 183 patients with AQP4-IgG-positive NMOSD,

MS and OND and healthy control samples were positive for MOG-IgG, and all 41 patients positive for MOG-IgG exhibited a clinical phenotype within the known clinical spectrum of MOG-IgG-related disease.

The female to male ratio was 28:13, and the mean age at onset of the disease was 28 years (range: 3 to 60 years). Clinical phenotypes at onset included optic neuritis (37%), myelitis (29%), brain attacks (24%), and poly-regional involvement (10%). During the mean prevalence period of 78 months (range: 3-298 months), 10 patients were monophasic and 31 patients was recurrent: 26/41 (63%) patients experienced at least one brain attack, 23/41 (56%) patients experienced at least one optic neuritis attack, and 20/41 (49%) patients experienced at least one myelitis attack. Of the 26 patients with brain attacks, 17 (65%) patients had ADEM-like attacks, 7 (27%) patients had fluffy brainstem attacks, and 2 (8%) patients had cortical attacks. Longitudinally extensive transverse myelitis was observed in 13 (65%) of 20 patients with myelitis, and bilateral involvement was observed in 8 (35%) of 23 patients with optic neuritis. At the last follow-up, simultaneous or sequential poly-regional involvement was observed in 25 (61%)/41 patients. The most common clinical presentation in these patients was a combination of optic neuritis and brain attacks (12/25 patients, 48%) followed by a combination of myelitis and brain attacks (7/25 patients, 28%) followed by a combination of optic neuritis and myelitis (3/25 patients, 12%). 3 (12%) patients

exhibited optic neuritis, myelitis and brain attacks, and the remaining 16 patients experienced isolated clinical syndromes, such as isolated myelitis (n=7), optic neuritis (n=5) and brain attacks (n=4).

2-3: Measurement of seropositivity by CBA-FACS

To further confirm the usefulness of IgG (H+L) in the FL-MOG-transfected stable cell line, experiments were performed using CBA-FACS on the sera of a total of 105/355 patients (65/105 patients were randomly selected, and 40 patients were MOG-IgG-seropositive) with CNS inflammatory disease and 25 patients with HC, and three different secondary antibodies IgG (H+L), IgG1-Fc and IgM were used (Figure 2A). The serostatus of 105 patients found by CBA-IF were classified as MOG-IgG-seropositive and seronegative, and the MFI ratio of each patient was determined by CBA-FACS. Using ROC curve analysis, the area under the curve for the MFI ratio of IgG (H+L) and IgG1-Fc was 1.00 and 0.97, respectively (Figure 2B).

The optimal cut-off was calculated from the ROC curve to detect seropositivity in 105 patients with CNS inflammatory disease, and the optimal cut-offs for anti-human IgG (H+L) and IgG1-Fc secondary antibodies were 3.4 and 2.3, respectively.

Diagnosis at last follow-up of 105 patients and seropositivity in each patient group were determined (Figure 3). All patients with AQP4-IgG-positive NMOSD, MS and OND were MOG-IgG-negative. On the one hand, the anti-human IgG1-Fc secondary

antibody detected MOG-IgG in 1 patient with AQP4-IgG-seropositive NMOSD and 2 patients with MS (specificity = 95%). The MFI ratio of the sera of all 25 patients with HC was below the cut-off for both IgG (H+L) and IgG1-Fc antibodies, and there were no IgM antibody-conjugated samples.

2-4: Correlation between CBA-IF scores and CBA-FACS

CBA-IF scores were determined as described above, and correlations between CBA-IF scores and CBA-FACS results were also determined. The IF scores of 40 MOG-IgG-seropositive patients were as follows: 10 patients scored 1 point, 13 patients scored 2 points, 10 patients scored 3 points, and 7 patients scored 4 points. The anti-human IgG (H+L) secondary antibody showed a stronger positive correlation with the CBA-IF score compared with the IgG1-Fc secondary antibody (Figure 4). (IgG (H+L): $r=0.87$, $p < 0.0001$ vs IgG1-Fc: $r=0.81$, $p < 0.0001$)

2-5: High concordance rate of Oxford CBA-IF and NCC CBA-IF

To further validate the analytic results, the serostatus of 125 patients (36 patients with AQP4-IgG-positive NMOSD, 13 patients with AQP4-IgG-negative NMOSD, 27 patients with MS, 21 patients with single or recurrent myelitis, 6 patients with single or recurrent optic neuritis, and 22 patients with ODD) was compared with IgG1 (Oxford) CBA-IF. Anti-human IgG (H+L) secondary antibody was used for (NCC) CBA-IF. As a result, both methods showed

consistent results for 122/125 (98%) samples ($\kappa = 0.919$, $p < 0.0001$), and it was confirmed in both methods that 21/125 patients were MOG-IgG-positive, and 101/125 patients were MOG-IgG-negative. Of 3/125 (2%) patients, 2 patients were positive only for (NCC) CBA-IF, and 1 patient was positive only for (Oxford) CBA-IF (Table 3). 2 patients who were MOG-IgG positive by (NCC) CBA-IF alone were patients with AQP4-IgG-negative NMOSD. The other sample (5 patients) was strongly positive for AQP4-IgG, borderline positive for MOG-IgG but positive for AQP4-IgG by (Oxford) CBA-IF, and negative MOG-IgG by (NCC) CBA IF.

【Table 3】

		NCC-CBA		Sum
		Positive	Negative	
Oxford-CBA	Positive	21	1	22
	Negative	2	101	103
Sum		23	102	125

($\kappa = 0.919$, $p < 0.0001$)

In addition, the present inventors found positive conjugation in MOG-transfected cells as well as EV-transfected cells by (NCC) CBA-IF and CBA-FACS (Figures 5A and 5B). MOG-IgG positive conjugation was observed for both anti-human IgG (H+L) and IgG-Fc secondary antibodies. The MOG-IgG MFI ratio was lower than the cut-off for all three secondary antibodies (anti-human IgG (H+L), IgG1-Fc and IgM) (Figure 5B).

From the above description, those of ordinary skill in the

technical art to which the present invention pertains will understand that the present invention may be implemented in other specific forms without changing the technical spirit or essential characteristics thereof. In this regard, it should be understood that the examples described above are illustrative and not restrictive in all respects. It should be construed that all changes or modifications derived from the meaning and scope of the claims to be described later rather than the detailed description and their equivalent concepts are included in the scope of the present invention.

WHAT IS CLAIMED IS:

【Claim 1】

A method of detecting myelin oligodendrocyte glycoprotein (MOG)-IgG from a sample, comprising the steps of:

(a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector;

(b) transfecting a cell with the MOG-cloned expression vector;

(c) culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line expressing MOG;

(d) incubating the selected cell line with a sample expected to contain MOG-IgG;

(e) adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and

(f) detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate.

【Claim 2】

The method of claim 1, wherein the nucleic acid encoding MOG consists of the base sequence as set forth in SEQ ID NO: 1.

【Claim 3】

The method of claim 1, wherein the secondary antibody is an anti-human IgG (H+L).

【Claim 4】

The method of claim 1, wherein the secondary antibody in step (e) does not show a cross reaction with IgM.

【Claim 5】

The method of claim 1, wherein the fluorescent protein is EGFP, GFP, YFP, DsRed2, Tdtomato, or mCherry.

【Claim 6】

The method of claim 1, wherein the cell is a human embryonic kidney (HEK) 293 cell, a HeLa cell, a Chinese hamster ovary (CHO) cell, or an NIH3T3 cell.

【Claim 7】

The method of claim 1, wherein the selection drug is hygromycin, puromycin, blasticidin, or G418.

【Claim 8】

The method of claim 1, wherein the sample is spinal fluid, blood or serum isolated from a patient with MOG-IgG-related central nervous system (CNS) inflammatory disease.

【Claim 9】

The method of claim 1, wherein step (f) is performing immunofluorescence assay or flow cytometry assay.

【Claim 10】

A method of providing information for prediction or diagnosis of MOG-IgG-related central nervous system inflammatory disease, comprising the steps of:

- (a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector;
- (b) transfecting a cell with the MOG-cloned expression vector;
- (c) culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line expressing MOG;
- (d) incubating the selected cell line with a sample expected to contain MOG-IgG;
- (e) adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and
- (f) detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate.

【Claim 11】

The method of claim 10, wherein the nucleic acid encoding MOG consists of the base sequence as set forth in SEQ ID NO: 1.

【Claim 12】

The method of claim 10, wherein the MOG-IgG-related central nervous system inflammatory disease is AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic

neuritis (ON).

【Claim 13】

A composition for diagnosis of MOG-IgG-related central nervous system inflammatory disease, comprising a stable transfected cell line expressing MOG, produced by the steps of:

(a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector;

(b) transfecting a cell with the MOG-cloned expression vector;
and

(c) culturing the transfected cell in a medium containing a selection drug.

【Claim 14】

The composition of claim 13, wherein the nucleic acid encoding MOG consists of the base sequence as set forth in SEQ ID NO: 1.

【Claim 15】

The composition of claim 13, wherein the MOG-IgG-related central nervous system inflammatory disease is AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic neuritis (ON).

【Claim 16】

A method of diagnosing MOG-IgG-related central nervous system

inflammatory disease, comprising the steps of:

- (a) cloning a nucleic acid encoding MOG into a fluorescent protein expression vector;
- (b) transfecting a cell with the MOG-cloned expression vector;
- (c) culturing the transfected cell in a medium containing a selection drug to select a stable transfected cell line expressing MOG;
- (d) incubating the selected cell line with a sample expected to contain MOG-IgG;
- (e) adding a fluorescence-conjugated secondary antibody to the incubated sample to form a MOG-IgG and secondary antibody conjugate; and
- (f) detecting or analyzing fluorescence from the MOG-IgG and secondary antibody conjugate.

【Claim 17】

The method of claim 16, wherein the nucleic acid encoding MOG consists of the base sequence as set forth in SEQ ID NO: 1.

【Claim 18】

The method of claim 16, wherein the MOG-IgG-related central nervous system inflammatory disease is AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), myelitis, encephalitis, or optic neuritis (ON).