Supplementary Methods:

Serum and CSF samples

A detailed clinical intake form was collected from outside investigators, summarizing the patient's neurological history, relapse features, neurological examination, MRI and CSF findings. For samples collected at the Brigham and Women's Hospital, the same information was obtained from the MS Center's clinical database. Adult and pediatric patients were diagnosed with relapsing-remitting MS according to the McDonald criteria[1]. Pediatric MS was defined as cases of MS with an onset of symptoms prior to 18 years of age. The definitions proposed by the International Pediatric MS Study Group [2] were used to distinguish ADEM, multiphasic ADEM and pediatric MS. Consistent with these definitions, the McDonald criteria were used to define Pediatric MS in all cases under the age of 18 years, including those under 10 years of age. ADEM was diagnosed in cases presenting with a polysymptomatic inflammatory demyelinating event, which included encephalopathy, seizure or coma. Subjects presenting with a first episode of an acute demyelinating event consistent with ADEM (such as acute onset, encephalopathy, seizures, coma) followed by a second demyelinating event were classified in a separate category (ADEM with relapse). One clinician validated each case included in this study using the data from the clinical intake form.

Viral encephalitis serum samples were provided by the New York State Department of Health. Sera from patients infected with West Nile virus or St. Louis Encephalitis virus were reactive in ELISA tests and were confirmed by cross species plaque reduction neutralization tests with paired acute and convalescent sera. Sera from patients with

enteroviral infection were collected on the same day as spinal fluids for which PCR tests

for enteroviruses were positive. Healthy control samples were collected at Brigham and

Women's Hospital from subjects self-reported to be free of MS or other autoimmune

disease. All serum and CSF samples were stored in aliquots at -80°C.

Flow Cytometry

   Stable transfectants that expressed full length MOG with a C-terminal green

fluorescent protein (GFP) domain and a control transfectant expressing only GFP were

generated in Jurkat cells. The entire coding sequence of human MOG was inserted into

the pEGFP.N1 vector (Promega) in frame with the first GFP codon. Jurkat cells were

transfected with MOG-GFP or pEGFP.N1 vector by electroporation and stable

transfectants were selected with G418 (2 mg/mL) in DMEM supplemented with 10%

fetal bovine serum, 2 mM L-glutamine, 100 µg/mL penicillin-streptomycin, and 35 mM

HEPES (Cellgro, Mediatech). Following drug selection, the brightest population of GFP-

expressing cells (~1%) was sorted on a FACS Aria flow cytometer (Beckton Dickinson)

for both MOG-GFP and GFP transfectants.

   A three-step staining procedure was used to enable sensitive detection of serum

antibodies to MOG. MOG-GFP and GFP transfectants (5×10^4 cells) were incubated with

serum (50 µl of a 1:50 dilution in FACS buffer, PBS + 2% BSA) in 96 well V-bottom

plates for 60 minutes at 4°C with gentle shaking and then washed twice with FACS

buffer. Secondary antibody (mouse anti-human, Fc specific, clone HP-6017, Sigma) was

diluted 1:1000 in FACS buffer and incubated with cells for 30 minutes at 4°C. Cells were

washed twice and incubated for 20 minutes at 4°C with streptavidin-PE (Molecular

Probes, Invitrogen) diluted to 1µg/mL. Following two washes, cells were resuspended in 200µL of 5% 7-AAD (BD Via-Probe) prior to data acquisition (10,000 events) on a BD FACS Calibur. Data were analyzed using FlowJo (TreeStar). Cells were gated on forward and side scatter, and 7-AAD positive cells were excluded. IgG positive gates were centered above the GFP-bright population, and positioned vertically such that <0.5% of cells from the GFP control transfectant were present in this gate.

Analysis of antibody binding by solid-phase ELISA

Human antibodies were detected by solid-phase DELFIA as previously described [3] using the human MOG extracellular domain refolded from inclusion bodies expressed in *E. coli*. Bound antibodies were detected with a biotinylated anti-human IgG and streptavidin-europium. Murine antibodies were detected in a similar manner, but with a biotin conjugated polyclonal anti-mouse Ig (Chemicon).

Experimental Autoimmune Encephalomyelitis

C57BL/6 mice were immunized s.c. with 100 µg of MOG (35-55) peptide (MEVGWYRSPFSRVVHLYRNGK) emulsified in CFA (Difco, Becton Dickinson) and injected twice intravenously with 150 ng/ml of pertussis toxin (List Biological Laboratories). Sera were obtained 33 days following immunization. Clinical assessment of EAE was performed daily and mice were scored for disease according to the following criteria: 0, no disease; 1, decreased tail tone; 2, hind limb weakness or partial paralysis; 3, complete hind limb paralysis; 4, front and hind limb paralysis; 5, moribund state.

Monoclonal Antibodies

Mice were immunized with recombinant human MOG extracellular domain expressed in *E coli* [3]. Hybridomas producing monoclonal antibodies to MOG were prepared from lymph nodes of immunized mice using standard protocols [4].

Supplementary References:

1.   McDonald, W.I. et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* **50**, 121-7 (2001).

2.   Krupp, L. International Pediatric MS Group: Consensus Definitions of Acquired Demyelinating Disorders of Childhood. *American Academy of Neurology Meeting* (2006).

3.   O'Connor, K.C. et al. Antibodies from inflamed central nervous system tissue recognize myelin oligodendrocyte glycoprotein. *J Immunol* **175**, 1974-82 (2005).

4.   Greenfield, E.A. et al. Monoclonal antibodies to distinct regions of human myelin proteolipid protein simultaneously recognize central nervous system myelin and neurons of many vertebrate species. *J Neurosci Res* **83**, 415-31 (2006).