

Live CBA

Briefly, human embryonic kidney 293 T (HEK293T) cells were transiently transfected with full-length recombinant human MOG α 1 isoform cDNA (plasmid encoding for full-length MOG tagged with an emerald green fluorescent protein (EGFP) or untagged; kindly donated by Prof Markus Reindl). The day after the coverslips were incubated with the patient serum, diluted 1:20, or CSF, diluted 1:2, in assay buffer (DMEM, 1% bovine serum albumin (BSA), and 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)), at room temperature (RT) for 45 min. In a subset of experiments, to confirm the negativity of IgG1 subclasses, serum was tested 1:10 and CSF, undiluted. After washing, cells were incubated with red-fluorescent mouse anti-human IgG-Fc γ -specific Ab (Jackson Scientific, cat#209-585-098), diluted at 1:750; at RT for 45 min, before washing and fixation with cold 4% paraformaldehyde for 5 min. Cells were washed with phosphate buffer saline (PBS), and mounted onto slides using a fluorescent mounting medium containing 1:1000 4',6-diamidino-2-phenylindole (DAPI, Dako). For initial MOG-IgG1 testing, cells were transfected with the untagged full-length MOG plasmid; in this case a green-fluorescent mouse anti-human IgG1-specific antibody (Invitrogen, cat#A-10631), diluted at 1:500, was used.

For further IgG subclasses, following the same steps as described above, mouse anti-human IgG1, IgG2, IgG3, and IgG4 secondary Abs (1:500; mouse anti-human IgG1 and mouse anti-human IgG4, mouse anti-human IgG3, Invitrogen, A10630, A10651, MH1031 respectively; mouse anti-human IgG2, ThermoFisher Scientific, 05-3500) were used, followed by red-fluorescent goat anti-mouse secondary Ab (1:1000).

Using a fluorescence microscope, binding was rated using a qualitative score from 0 to 4, according to the intensity of the staining, and considering scores ≥ 1 as positive values. Endpoint titrations were performed starting at 1:20, with 1:2 dilution steps. The endpoint titer was considered as the last dilution showing cell surface fluorescence. The cut-off for positivity was 1:160 for IgG (Fc γ), and 1:20 for the IgG subclasses.