Supplementary data

Live cell-based assays (Plasmids were provided by Markus Reindl, Innsbruck, Austria).

*Assay of MOG in Lyon*

HEK293 cells were transfected with pEGFP-N1-hMOG plasmid After 48 hours, transfected cells were dissociated with Accutase (Sigma-Aldrich, A6964) and incubated with phosphate-buffered saline 8% normal goat serum for 30 minutes at room temperature (RT). Then, patient’s serum diluted at 1:640 was incubated with transfected cells for 30 minutes at 4°C. This cut-off was selected to avoid false-positive signal detected with healthy control in previous studies.1 Cells were fixed with 1% paraformaldehyde for 15 minutes and then incubated 20 minutes at RT in the dark with a secondary antibody Allophycocyanin-Goat anti human IgG-Fcγ fragment-specific (1:100 dilution, Jackson ImmunoResearch 109-136-170). Evaluation of signal intensity was performed with CANTO II flow cytometer (Becton Dickinson). When positive, a titration of samples was performed by serial dilution from 1:320 to 1:40.960.

*Assay of MOG in Barcelona*

Ten randomly selected samples and the two MOG-Ab positive samples from Lyon were also examined for MOG-IgG in Barcelona, using an in-house CBA with HEK293 cells transfected with the full-length MOG C-terminally fused to EGFP as reported (*Sepúlveda M et al., 2016. J Neurol 2016*). The live-cells were incubated at 37 °C with serum (1:160 diluted with DMEM for IgG) for 35 min. After removing the media and washing with PBS, HEK cells were fixed with 4% PFA for 10 min and incubated with 0.3% Triton X-100 for 5 min. Then cells were immunolabeled with Alexa Fluor 594 secondary antibody against IgG-Fcɣ fragment-specific (1:1000). A cut-off ≥1:160 was considered as MOG-IgG positive sample. Evaluation of signal was performed by immunohistochemistry.

Supplementary Figure e1

Figure e1. Disease course in the two Multiple Sclerosis patients with MOG-Ab-positivity