Determination of human anti-MOG antibodies binding patterns in optic nerves
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Research question and background
Optic neuritis (ON) is an inflammatory disease of the optic nerve, manifested with pain and visual loss, often associated with multiple sclerosis or with Neuromyelitis Optica Spectrum Disorders (NMOSD). Recent evidence suggests that certain forms of optic neuritis are associated with anti- myelin oligodendrocyte glycoprotein (MOG) antibodies. A distinct clinical subset of optic neuritis is characterized by multiple episodes, restricted to one or both optic nerves, which occur within months or weeks, without any other associated clinical or radiological findings. This entity, defined either as recurrent optic neuritis (rON) or as Chronic Relapsing Inflammatory Optic Neuritis (CRION) is typically corticosteroid-responsive and corticosteroid-dependent, often requiring immunosuppressive therapy for corticosteroid-sparing effect. Our aim was to determine whether AQP4-negative patients with optic neuritis harbor antibodies to MOG and whether anti-MOG antibodies are clinically relevant.

Methods and tissues used
We tested sera from 111 patients with clinical evidence of at least one episode of unilateral or bilateral optic neuritis. The patients, referred to our diagnostic service for AQP4 testing, were all APO4-negative. 12 Primary Progressive MS and 30 Relapsing Remitting MS patients were used as disease controls. The Ethics Committee of the University of Athens granted ethical approval. Anti-MOG screening was performed using a cell-based assay (CBA). Patient sera (1:60 dilution) were applied on live human embryonic kidney (HEK) 293T cells, transiently transfected with full-length MOG-EGFP, followed by a goat-anti-human secondary antibody. To investigate optic nerve specificity, anti-MOG-positive sera and IgG were applied onto 10 μm non-fixed or 2% paraformaldehyde-fixed sections of fresh-frozen human optic nerves (Netherlands Brain Bank).

Results and conclusion
Anti-MOG antibodies were detected in 8 patients with ON; 5 of them were diagnosed as definite rON because they had ≥3 episodes of ON without any other clinical symptomatology. Brain/orbital MRI or CSF analyses (when available) were normal except for optic nerve enhancement in two of them. All anti-MOG-positive patients were steroid responsive but two of them, required other immunosuppressants, plasmapheresis or IVIG to suppress or prevent relapses. Furthermore, no specific binding of the patients’ serum or IgG was observed in optic nerve tissue preparations (Figure 1). Patients with the clinically distinct entity of rON (AQP4-negative) are
characterized by antibodies against MOG. Because anti-MOG antibodies have been also detected in typical NMOSD (either AQP4-negative or AQP4-positive), different MOG epitopes may distinguish rON from NMOSD. Whether in rON patients the anti-MOG antibodies are causally connected to conduction block in the optic nerve or demyelination is unclear.

**Figure 1.** Staining of human optic nerve. Fresh-frozen human optic nerve was stained with either serum of purified IgG from patients with recurrent optic neuritis. The tissue was either stained non-fixed or fixed with 2% paraformaldehyde for 5min in order to reserve epitope integrity. A representative section is shown in panel A where no specific binding can be observed. In the same section, an anti-MOG monoclonal was applied, see panel B. A specific pattern of binding in oligodendocyte processes can be observed. The overlay in panel C shows the absence of any co-localization.