Supplementary Appendix e-1

Flow cytometry assay for AQP4 antibody detection

For construction of the recombinant lentiviral vectors, the ORF of EGFP gene was amplified from pEGFP-N1 plasmid using the designed primers: EGFPF: 5'- CTAGGATCCATGGTG AGCAAGGGCGAGGAG-3', EGFPR: 5'- CAG GTCGACGAATTCGCGGCCGCCTCGAGCTGCAGT TACTTGTACAGCTCGTCCATGCCG-3'. The product was purified, sequencing-confirmed and used to replace the EF1-CopGFP sequence in the lentiviral vector pCDH-CMV-MCS-EF1-CopGFP. AQP-4 (M1) was amplified using cDNAs from human brain tissues as the template with the following primers: AQP4F: 5'-ACTTCTAGAATGAGTGACAGACCCACAGCAAG -3', and AQP4R: 5'-CCAAGATCTTGAACCGCCTCCACCTACTGAAGACAATA CCTCTCCAGATTG-3'. The purified PCR product was sequencing confirmed and inserted into the pCDH-CMV-MCS-EGFP plasmid to get an AQP4 (M1)-EGFP expressing lentiviral vector. The constructed lentiviral vetor combined with three helper plasmids pLP1, pLP2 and pMDG were used for virus packaging using Lipofectamine 2000 as transfection reagents according to the manufacture instructions. Infectious lentiviruses were harvested at 48 hours post-transfection and then concentrated and tittered. Then the 293T cells were seeded onto 6-well plates at a density of 5×10^5/well, the 2×10^6 IU viruses per well of viruses were used to infect 293T cells with 8 g/ml polybrene for 3 hours. Then the virus soap was replaced by fresh DMEM complete medium for propagation. Two weeks later, the stable transfected 293T cells were sorted by FACS according to EGFP intensity. The sorted cells were then propagated and passaged for detection using. For Flow cytometry assay, AQP4-M1-EGFP/293T cells were incubated with

diluted human sera (1/10) in FACS buffer (PBS containing 1% BSA and 0.03% NaN3) for half hour, EGFP/293T cells were used as control. After washing, the cells were incubated with PerCP-Cy5.5-conjugated goat anti-human IgG Ab (Biolegend, U.S.A). Half an hour later, the cells were washed twice with FACS buffer and then analyzed on a FACS Calibur (BD Biosciences), and PerCP-Cy5.5 mean fluorescence intensity (MFI) was measured. MFI value for PerCP-Cy5.5 corresponding to IgG bound to AQP4-EGFP-transfected cells was compared with the MFI value for that patient's IgG binding to EGFP-transfected control cells in the same aliquot. Resulting ratios (MFI AQP4-EGFP-transfected cells/MFI EGFP-transfected cells) were reported as AQP4-IgG Binding Index; values of ≥3 were considered positive. Subsequently, these results of AQP4 antibody assay with in-house FACS was verified by a visual fluorescence-observation cell-based assay that incorporated HEK293 cells transfected with human M23 isoform (Euroimmun, Lübeck, Germany) according to the manufacturer's instructions.