ANTIBODIES TO AQUAPORIN-1 ARE NOT PRESENT IN NEUROMYELITIS OPTICA

Although more than 70% of all patients with neuromyelitis optica (NMO) are seropositive for aquaporin-4 (AQP4) antibodies, a substantial proportion of patients fulfilling the clinical criteria for NMO or limited forms of the disease (NMO spectrum disorders [NMOSD]) are negative for these antibodies. This raises the possibility of other autoantibodies in these patients. One putative target, the ubiquitously expressed water channel aquaporin-1 (AQP1), which is partially lost or internalized in certain NMO lesions, was described in a subset of patients with NMOSD. However, AQP1 antibodies were also found in patients with multiple sclerosis (MS), thus raising concerns about the specificity of these findings. Therefore, we developed a recombinant live cell immunofluorescence assay (CBA) for AQP1 antibodies based on our AQP4 antibody assay. We analyzed 176 serum samples from Austrian patients with NMOSD (n = 67), Austrian patients with MS (n = 31), and controls (n = 78) for the presence of IgG AQP1 antibodies and AQP4 antibodies. Furthermore, both assays were validated in a blinded cohort of 58 patients with NMOSD (n = 36) or MS (n = 22) from Lyon (France) and Oxford (United Kingdom). Clinical and demographic data of patients and controls are shown in the table.

Methods. Analysis of AQP1 antibodies and AQP4 antibodies was performed using a live CBA described previously. Briefly, HEK293A cells were transiently transfected using the pcDNA6.2C-EmGFP-GW/TOPO plasmid (Invitrogen, Carlsbad, CA), expressing AQP4 (isoform M23) or AQP1 (isoform 1) fused C-terminally to emerald green fluorescence protein. Transfected cells were blocked with goat IgG in phosphate-buffered saline (PBS)/10% fetal calf serum (FCS) (Sigma-Aldrich, St. Louis, MO) followed by serum diluted 1:20 and 1:40 in PBS/FCS for 1 hour at 4°C. Serum preabsorption with liver powder was not performed because a previous report indicated loss of AQP1 antibodies after pretreatment. Bound antibodies were detected using Cy3Tm-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 minutes at room temperature. Bound antibodies were determined using a fluorescence microscope (Leica DMI 4000B). All samples were evaluated by 2 independent, clinically blinded investigators who agreed on all samples.

Standard protocol approvals, registrations, and patient consents. The present study was approved by the ethical committee of Medical University of Innsbruck (#AM3041a), the Oxfordshire Research Ethics Committee (#10/H0606/56), and the French data protection authority.

Results. Expression of AQP1 was verified by staining with a commercial AQP1-specific antibody recognizing full-length AQP1. However, the antibody only recognized the target after fixation of AQP1-transfected HEK293A cells with paraformaldehyde (figure e-1A at Neurology.org/nn). In order to prove surface expression and correct topology of AQP1, a myc-tag was inserted at position T120 (extracellular loop C) by site-directed mutagenesis. Staining with an anti-myc-tag monoclonal antibody clearly showed the surface expression of AQP1 in live HEK293A cells (figure e-1B).

As seen in the table, AQP4 antibodies were detected in 81 of 103 (79%) patients with NMOSD in the combined cohorts. AQP4 antibodies were absent in 53 patients with MS and 78 controls; therefore, the specificity of the AQP4 antibody assay was 100%. In contrast, AQP1 antibodies were absent in all 234 serum samples from patients with NMOSD and MS and controls. Although the AQP4 antibody CBA showed high sensitivity and specificity, a comparable AQP1 antibody CBA did not detect any antibodies in 234 serum samples (figure e-1C).

Discussion. This finding is in contrast to previous studies by 2 groups reporting the presence of AQP1 antibodies in 17%–74% of patients with NMOSD, but also in controls. In our opinion, these differences could be explained by methodologic differences between the studies (using radioimmunoprecipitation, ELISA with peptides, or fixed CBAs might have exposed intracellular epitopes, whereas our assay only detects antibodies to extracellular epitopes). Methodologic differences have proven to be a constant problem in this field for decades. For example, there is now clear evidence that antibodies to the myelin oligodendrocyte glycoprotein are specific
for a subset of demyelinating diseases only if appropriate CBAs are used (these antibodies are detected at similar frequencies in patients and controls using ELISA). Similarly, although numerous studies confirm the importance of AQP4 antibodies as diagnostic biomarkers for NMOSD, the seropositivity rates are influenced by the presence of AQP4 antibodies as diagnostic biomarkers for NMOSD. To conclude, our study fits very well with AQP1 expressed in a complex against which the immune system recognizes absent in NMOSD. To conclude, our study fails to confirm the presence of AQP1 antibodies in NMOSD.

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