Reply to Rossi et al.: Immunohistopathological findings in neuromyelitis optica concur with immunobiological observations in vitro

Aquaporin-4 (AQP4) water-channel–specific IgG unifies a spectrum of autoimmune astrocytopathies exemplified by neuromyelitis optica (NMO): relapsing inflammatory myelopathy, optic neuritis, and disorders affecting AQP4-rich circumventricular organs. AQP4-IgG detection in serum distinguishes these disorders from other inflammatory demyelinating disorders. The points of our study (1) were to emphasize that (*i*) IgG-AQP4 interaction has multiple molecular sequelae that plausibly account for diverse pathology (edema, inflammation, demyelination, and necrosis) and (*ii*) some outcomes are AQP4 isoform (M1 or M23)-specific. Our study involves immunocytochemistry and freeze-fracture EM of cultured rodent astrocytes, HEK293 cells transfected with non-tagged human AQP4s, *Xenopus* oocytes expressing GFP-tagged human AQP4s, and NMO patient brain immunohistopathology. Because serum IgG is polyclonal, we used high-titered sera (or IgG) pooled from at least 50 patients with NMO (binding > 1,000 nmol AQP4/L) to broadly investigate potentially pathogenic, AQP4-specific outcomes of IgG-AQP4 interaction in live cells. The correspondents question our data's validity (2), citing conflicting data they obtained by using various AQP4 isoform-transfected cell types, sera from six individual patients with NMO, and mouse neuropathology.

(a) The correspondents challenge our evidence for differential internalization of M1-AQP4. M1 internalization is readily demonstrable in HEK293 cells (1, 3), oocytes (figure 5b in ref. 1), and astrocytes [figure 2 c and d in ref. 1; and refs. 4, 5 (isoform unidentified)]. Our investigation of NMO-IgG interaction with M23 (1) demonstrated that M23 high-order arrays (i.e., ultrastructural specialization of astrocytic membranes facing sites of rapidly fluxing water and K^+ ions) internalize relatively slowly and incompletely in HEK293 cells (figure 2a in ref. 1). In cultured astrocytes (figure 3 in ref. 1), we quantified NMO-IgG-dose–dependent coalescence of remaining surface M23 into larger orthogonal arrays by blinded freeze-fracture EM (the gold-standard technique in this field).

(b) The correspondents misunderstood our conclusions regarding complement activation. Their own observation (2) confirms our report that M23-bound IgG activates complement more effectively than M1-bound IgG (1). The unappreciated point is that the quiescent CNS lacks ambient complement. When it has been perturbed, complement secreted locally would be activated explosively by IgG persisting on residual noninternalized M23, because Fc-domains displayed on AQP4 lattices would precisely accommodate the requisite C1q complement component (predicted in ref. 3).

(c) The correspondents challenge our concept and evidence for impairment of water fluxes by NMO-IgG. Disruption of water homeostasis, caused by endocytosis or direct channel blockade, is an anticipated outcome of IgG-AQP4 interaction. Selective slowing of *Xenopus* oocyte swelling by NMO-IgG at 4 °C precludes endocytosis (figure 5 in ref. 1), implying that an antigen-binding domain of IgG accommodating ∼7 aa, and of appropriate epitope specificity, can partially occlude one or more water pores on an AQP4 tetramer. The low sensitivity of oocyte lysis for assessing water permeability (2003 Nobel Prize; ref. 6) emphasizes the effectiveness of an appropriate NMO-IgG specificity [water flux reduced ∼1.5 fold (>140% control volume); figure 5e in ref. 1].

(d) The correspondents challenge our immunohistopathological findings in patients' brain tissues. AQP4 protein is recognized to be up-regulated in a number of neuropathological states (e.g., ischemia, tumor, bacterial meningitis, contusion). Our study illustrates, in autopsied brain of two patients, NMO-specific features characterized by sublytic astrocyte injury with incomplete internalization of AQP4 within edematous regions otherwise devoid of AQP4. Although these findings contradict what is seen in mice acutely injected with NMO-IgG, intracerebrally, animal data must ultimately be reconciled with the characteristic neuropathological findings of the human disease, rather than vice versa. We agree that further investigation is required.

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Conflict of interest statement: V.A.L. is a named inventor on a patent relating to AQP4 as a target of pathogenic autoantibodies in NMO and related disorders and on a pending patent related to AQP4 applications to cancer; has received greater than the federal threshold for significant interest from licensing of this technology; and receives no royalties from the sale of Mayo Medical Laboratories' service serological tests. However, Mayo Collaborative Services, Inc., receives revenue for conducting these tests. In addition, V.A.L. and S.R.H. are named inventors on two patent applications filed by the Mayo Foundation for Medical Education and Research relating to functional assays for detecting NMO/AQP4 antibody.

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