Illuminating neuromyelitis optica pathogenesis

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What is the most important moment in the history of a disease? Let us consider neuromyelitis optica (NMO), which was coined by French neurologist Eugene Devic in 1894 for a disease believed until recently to be a subtype of multiple sclerosis (MS). The discovery (in 2004) of a disease-specific biomarker for NMO revolutionized our understanding of both NMO and MS (1). In PNAS, Hinson et al. (2), including Lennon, who discovered the biomarker, address mechanisms of pathogenesis mediated by that same biomarker.

NMO and MS, clinically characterized by the French neurologist Jean-Martin Charcot in 1868, have long been intertwined (3). NMO and MS are both inflammatory diseases of the CNS. MS, the prototypic demyelinating disease, causes selective destruction of CNS myelin, a fatty membrane that nourishes and insulates nerve fibers. Early symptoms of MS arise because demyelinated nerve fibers conduct impulses poorly, and later symptoms may involve degeneration of nerve fibers deprived of myelin's metabolic support. NMO seemed to be more destructive than MS, and it remained unclear whether selective demyelination could be seen. However, clinical similarities were striking: both MS and NMO showed onset around 30–40 y, and women were more proportionally affected than men. Both exhibited years of clinical quiescence punctuated by abrupt attacks of neurological symptoms that variably receded after weeks to months. Both typically impaired vision and walking among other functions. NMO, 10-fold less common than MS in North America and Western Europe, was, therefore, considered a subtype of MS. There were differences: the eponymic effects of NMO seemed focused specifically on optic nerves and spinal cord, whereas MS was less commonly fatal and showed more widely distributed symptoms.

Among susceptible, largely Caucasian populations, MS cases are concentrated in temperate zones of both hemispheres. Worldwide, about 2 million people are affected by MS. Western NMO research has lagged behind MS studies, whereas Asian research on NMO, previously termed opticospinal MS, was vigorous but suffered from the relatively low disease prevalence. With recognition that NMO, unlike MS, occurs equally in all ethnic groups and climates, it seems likely that the global case burden of NMO may approach one-quarter to one-half the case burden of MS, albeit much more widely dispersed (4). NMO, previously thought a rare disease, is now considered a legitimate target for drug development.

A Diagnostic Biomarker for NMO

A biomarker is a quantifiable component that indicates the presence of a specific disease. The NMO biomarker is an antibody to aquaporin-4 (AQP4), a water channel found on CNS astrocytes (glial support cells for neurons) as well as in kidney and skeletal muscle (5). Its discovery entailed the opportune confluence of research projects at the Mayo Clinic. Neurologists Brian Weinshenker and Dean Wingerchuk accumulated a multicenter NMO clinical cohort with standardized diagnostic criteria (6). Their initial studies elucidated that NMO patients, unlike those patients with MS, often have other diseases (such as lupus) typified by production of antibodies to host tissues. Neurologist Claudia Lucchinetti found consistent evidence suggesting antibody-mediated damage in NMO tissues (7). Neuroimmunologist Vanda Lennon conducts basic research and directs a reference laboratory, where she diagnoses disease and also observes and catalogs unusual patterns of serum antibody binding to neural tissues. Together with her clinical colleagues, Lennon showed that 55% of NMO cases contained a serum antibody that exhibited a distinctive brain tissue binding pattern (1). No MS cases or controls showed this binding pattern. Intrigued, Lennon contacted a dozen individuals (of >80,000 tested at the Mayo reference laboratory over the years) with sera that contained antibodies with this unique brain tissue binding pattern. Amazingly, 10 of 12 (all who could be contacted) had NMO symptoms. In 2004, the mystery was solved: NMO was a distinct disease that was different from MS and characterized by the presence of a unique serum antibody (1).

The present results draw a tighter circumstantial noose around AQP4 antibodies as pathogenic elements in NMO.

Distinguishing MS from NMO took 105 y, but only 1 y was required to establish its molecular pathogenesis: the antibody was found to bind AQP4, the major CNS water channel (8). Astrocytes express AQP4 on extensions termed endfeet that abut cerebral vessels. The AQP4 antibody was the first biomarker for an autoimmune CNS disease unrelated to systemic cancer. NMO is now recognized as a disease that initially affects astrocytes, with a unique biology reflecting the functional attributes of the target cell. MS is a disorder of myelin and oligodendrocytes, whereas NMO is a condition affecting astrocytes. Newer molecular tests for AQP4 antibodies show that roughly 80% of NMO cases are antibody-positive (9).

Are AQP4 Antibodies Pathogenic?

The next and crucial question was whether the antibody was pathogenic or merely correlative. Circumstantial evidence suggested the former explanation. A procedure termed plasma exchange, which removes plasma proteins from the circulation, often terminated NMO attacks (10). AQP4 protein was selectively depleted from NMO but not MS spinal cord autopsy tissue sections (11). AQP4 antibody-rich plasma from patients modulated the protein from the surfaces of AQP4-transfected cells (12). Arguing against pathogenicity for AQP4 antibodies, patients were shown to have circulating AQP4 antibodies for several years before onset of disease, and mice engineered to express high titers of AQP4 antibodies failed to develop NMO-like pathology (13). Direct cerebral injections of AQP4 antibodies along with human complement produced tissue destruction reminiscent of the most aggressive lesions, but results from this model system were difficult to interpret in the context of the spontaneous disease, which is associated mainly with antibodies in plasma (13).

The present results draw a tighter circumstantial noose around AQP4 antibodies as pathogenic elements in NMO (2). One conundrum about AQP4 regards the physical state of the protein in astrocytic endfeet. Freeze-fracture electron micrographic studies from the 1970s showed patterned structures termed

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orthogonal array particles (OAPs) on the astrocytic endfeet, and these OAPs were later shown to consist of AQP4. Furthermore, the assembly of OAPs was enabled by differential splicing of AQP4 mRNA, yielding two major isoforms termed M1 and M23. The shorter M23 isoform packed more tightly into OAPs. Previous work from Hinson et al. (14) showed that AQP4 antibodies removed critical functional astrocytic components from the endfeet along with AQP4. Most importantly, the astrocyte glutamate transporter EAAT2, found in a complex with AQP4 on the membrane, was removed by the AQP4 antibodies. Lack of membrane EAAT2 could plausibly lead to accumulation of glutamate in the extracellular fluid to levels toxic for myelin-forming oligodendrocytes, producing a secondary loss of myelin (14).

The paper in PNAS (2) addresses additional downstream effects of binding AQP4 antibodies and confronts a key riddle of NMO pathology: if AQP4 is assembled entirely in OAPs, how can AQP4 antibodies remove the protein from the plasma membrane? The proposed solution is ingenious: that AQP4 antibodies see the same structure on M1 and M23 isoforms of AQP4, which are predicted to have identical extracellular domains. AQP4 antibody binding to M1 compared with M23, however, is suggested to exert different consequences. The work by Hinson et al. (2) reports a membrane-associated pool of AQP4 mainly composed of M1 and capable of water channel function but largely unassembled in highly-ordered and stable OAPs. M1 can, therefore, be removed from the plasma membrane without disrupting OAPs and without activating cell-killing complement factors. The outcome of AQP4 antibody binding to M1 isoform is an astrocyte that survives and exhibits OAPs but is functionally impaired. Because astrocytic AQP4 channels maintain tissue water homeostasis, edema could be predicted to result and would affect myelin integrity. Supporting the disease relevance of these in vitro studies, Hinson et al. (2) describe tissue changes in NMO autopsy CNS sections compatible with their results: morphologically viable astrocytes that lack AQP4 with adjacent abnormally swollen, vacuolated myelin, consistent with effects of edema. Conversely, binding of AQP4 antibodies to M23-enriched OAPs leads to activation of complement, which would likely produce astrocyte-killing and tissue necrosis, the more dramatic pathology described in NMO studies. Taken together, the correspondence between in vitro models of NMO pathology and in situ tissue changes in the work by Hinson et al. (2) produces a compelling account of NMO pathogenesis produced by AQP4 antibodies. The story is not entirely concluded, of course, because findings from the varied laboratories studying NMO have been discordant with regard to functional outcomes of antibody binding. The achievement of the paper by Hinson et al. (2) is to show how in vitro results can guide the neuropathologist to unnoticed tissue changes to illuminate pathways of disease causation. The hypotheses generated by these findings will lead directly to additional testable predictions and ultimately, improved understanding and treatment of this often severe disease.

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