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Neuromyelitis Optica: Deciphering a Complex Immune-Mediated Astrocytopathy

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NEUROMYELITIS OPTICA: A NOVEL AUTOIMMUNE CENTRAL NERVOUS SYSTEM DISORDER

In 1894, Eugene Devic (1) and Fernand Gault summarized multiple cases of acute, concurrent optic neuritis (ON) and transverse myelitis (TM), which they termed “neuromyéélite optique aiguë.” Over the ensuing decades, the publication of multiple additional cases of neuromyelitis optica (NMO) documented that this relatively rare condition had both relapsing and monophasic presentations. Central nervous system (CNS) injury was usually dramatic: severe myelitis that was accompanied, preceded, or followed by severe vision loss progressing to complete blindness (2). Some early clinicopathologic studies argued for a distinct nosology, whereas others suggested classification with disseminated sclerosis (multiple sclerosis [MS]) or diffuse sclerosis (Schilder disease). During this time, it is likely that definitive categorization of NMO was hampered by both the overlapping clinical presentations and histopathology of demyelinating disorders.

Although evidence for humoral-mediated disease pathology was observed in active NMO lesions (3), it was the identification of an autoantibody against the aquaporin-4 (AQP4) water channel in NMO patients that dramatically changed perspectives on the pathophysiology of disease and the relationship between NMO and other inflammatory CNS disorders (4,5). However, several fundamental questions needed to be addressed. First, are AQP4 autoantibodies (AQP4-IgG) causing pathology or are there additional NMO-specific immune targets? Second, how does an immune response against AQP4, the major astrocytic CNS water channel, result in myelin destruction? Third, why are the optic nerves and spinal cord predisposed to injury while AQP4-expressing tissues such as lung, stomach, kidney, and skeletal muscle spared? And finally, how can we use our understanding of disease mechanisms in NMO to preserve vision and neurologic function? In this review, we will assess progress in the field toward answering these important questions and examine how information from the bench is leading to the generation of novel therapeutic strategies at the bedside.

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AQP4-IgG: A SPECIFIC AND PATHOGENIC HUMORAL AUTOANTIBODY

In 2004, Lennon et al (5) identified an autoantibody in NMO patient sera, termed NMO-IgG, that stained CNS microvessels, pia, subpia, and Virchow–Robin spaces in mouse midbrain and spinal cord by indirect immunofluorescence (IIF). Serum samples from NMO (5), MS, Japanese optic–spinal multiple sclerosis (O-S-MS), recurrent ON or TM, and various immune, vascular, nutritional, neoplastic, paraneoplastic, and idiopathic conditions were subsequently assayed for this immunoreactivity. NMO-IgG showed high sensitivity (73%; confidence interval [CI]: 60%–86%) and specificity (91%; CI: 79%–100%) for NMO patients. Interestingly, O-S-MS demonstrated similar sensitivity (58%; CI: 30%–86%) and specificity (100%; CI: 66%–100%), and 46% of cases of recurrent ON and TM cases were also positive for NMO-IgG. None of the classical MS or miscellaneous control samples were positive. The similar frequency of positive samples among NMO and Japanese O-S-MS patients also suggested that these 2 disorders were identical and that a significant fraction of individuals with recurrent ON and TM at high risk for NMO could be identified by IIF testing. The target of the serum autoantibody was rapidly identified as the AQP4 water channel (4), the unique CNS staining pattern on IIF resulting from localized expression of AQP4 on astrocyte foot processes on the abluminal face of CNS microvessels and pia mater.

The identification of AQP4 as a specific immune target in NMO allowed the further development of specific quantitative and semiquantitative immunoassays for detection of AQP4-IgG in body fluids. Enzyme-linked immunosorbent assay (ELISA), fluorescence immunoprecipitation assay (FI-PA) and radioimmunoprecipitation assay, and cell binding assays (CBA) with fluorescence microscopy or fluorescence-activated cell sorting (FACS) detection were subsequently developed and used to independently verify the sensitivity and specificity of AQP4-IgG for NMO (6,7). Although the number of studies and subjects varied considerably, they consistently demonstrated that AQP4-IgG was sensitive (range: 49%–77%) and specific (range: 96%–99%) for NMO (7). A multicenter comparison of IIF, ELISA, FIPA, CBA, and FACS assays demonstrated that CBA and FACS assays were most sensitive; IIF and FIPA assays lacked sensitivity (8). Therefore, multiple independent studies confirmed AQP4-IgG as a disease-specific biomarker of NMO and indicated that cell binding assays offer optimal sensitivity and specificity.

Disease-specific autoantibodies, however, are not necessarily pathogenic. In NMO, the pathogenicity of AQP4-IgG was examined using multiple experimental strategies. Serum NMO-IgG, patient-derived AQP4-specific monoclonal recombinant antibodies (rAbs), or control human IgG were administered to rats with experimental autoimmune encephalomyelitis (EAE), and the CNS was examined for NMO-specific histopathology (9–11). NMO-IgG or patient-derived AQP4-specific rAb, but not control serum or control rAb, caused NMO-like pathology in the background of myelin-targeted EAE. CNS lesions demonstrated perivascular AQP4 and astrocyte loss, IgG and complement deposition, granulocytic and lymphocytic infiltrates, macrophage influx, and myelinolysis. NMO pathology was specific to NMO-IgG or AQP4-specific rAb. Using a distinct model of direct NMO-IgG and human complement (HC) intracerebral injection (ICI), Saadoun et al (12) were able to recapitulate the seminal histopathologic features of NMO lesions independent of a systemic immune response. Identical histopathology could be produced in the ICI

model using AQP4-specific rAb and HC (13), indicating that intracerebral complement activation by AQP4-specific IgG is sufficient to induce the seminal features of NMO histopathology (Fig. 1).

Are There Other Target Antigens in Neuromyelitis Optica?

Approximately 25% of patients meeting the 2006 Wingerchuk diagnostic criteria (14) for NMO are seronegative for AQP4-IgG (8). A small fraction of these seronegative patients have serum myelin oligodendrocyte glycoprotein autoantibodies (MOG-IgG) (15). AQP4-IgG and MOG-IgG are rarely observed in the same patient (16,17). Despite overlapping clinical presentations, multiple lines of data indicate that AQP4-IgG and MOG-IgG seropositive patients are distinct demyelinating conditions (18,19). Although both MOG-IgG and AQP4-IgG seropositive patients have relapsing clinical courses with significant disability (20), a lower female bias and higher predominance of monophasic disease are reported in multiple MOG-IgG-positive cohorts (18,19,21). In addition, radiographic features often distinguish MOG-IgG seropositive TM and ON from their AQP4-IgG counterparts: inflammation of the conus and cauda equina, perineuritic enhancement of the optic nerve, and shorter optic nerve lesion length (20,22). Moreover, functional recovery and steroid responsiveness are more frequently reported for MOG-IgG seropositive ON (18,19,21,23,24). Importantly, the histopathology of brain lesions from MOG-IgG seropositive patients reveal Type 2 MS pathology (25), and MOG-IgG does not produce NMO lesion pathology in the ICI animal model (26,27).

In recognition of the broadening clinical presentation of NMO, the International Panel for NMO Diagnosis recently established new criteria for the diagnosis of neuromyelitis optica spectrum disorder (NMOSD) (28). Novel clinical and radiologic criteria for seronegative NMOSD were formulated, and in a recent analysis of a large cohort of NMOSD patients, the fraction of AQP4-IgG seronegative patients fell from 15% to 10% (29). The remaining small fraction of AQP4-IgG seronegative NMOSD patients may be due to limited assay sensitivity (6–8), AQP4-IgG seroconversion (29), and clinically overlapping demyelinating conditions (15,19,30). The possibility of a pathogenic T-cell-restricted adaptive immune response against AQP4 is unlikely given the inability to reproduce complete AQP4-targeted pathology following the adoptive transfer of AQP4-specific T cells in experimental systems (31,32). Although additional autoantibodies are frequently observed in AQP4-IgG seropositive and seronegative NMOSD patients (33,34), candidate autoantigen targets, such as aquaporin-1, have failed to show reproducible disease association (35,36).

NMO LESION PATHOLOGY: LINKING ASTROCYTE DESTRUCTION TO DEMYELINATION

Myelinolysis Precedes Demyelination in NMO Lesions

Understanding the link between AQP4-IgG-mediated astrocyte injury and downstream oligodendrocyte loss, myelin destruction, and neuronal injury may reveal critical stages for interventions to prevent or ameliorate CNS injury (Fig. 2). Early NMO lesions exhibit regions of intact myelin associated with either complete astrocyte loss or significantly reduced AQP4 and glial fibrillary acidic protein (GFAP) staining (37,38). The remaining

astrocytes appear fragmented with surrounding GFAP-positive debris and perivascular GFAP-laden macrophages (38). Perivascular immunoglobulin and terminal complement complex (C5b-9; C9neo) deposition are evident, consistent with the primary humoral immune-mediated destruction of perivascular astrocytes (38,39). Myelin in these regions show numerous intracytoplasmic vacuoles or widening of the extracellular space between myelinated fibers, and immunohistochemical stains reveal early loss of myelin-associated glycoprotein (38,39). Oligodendrocytes are either reduced in number or absent, and many remaining oligodendrocytes demonstrate seminal features of apoptotic cell death including nuclear chromatin condensation (38), immunoreactivity for activated caspase-3 (38), and DNA fragmentation (39). Regions of demyelination in active NMO lesions are marked by the presence of lipid- and complement-laden macrophages (38,39). In contrast to still-myelinated areas, active demyelinating regions displayed more abundant infiltrating immune subsets, such as granulocytes, macrophages, eosinophils, lymphocytes, and plasma cells (38,40–42).

Regions of asynchronous glial injury in human NMO lesions provide evidence that targeted destruction of CNS astrocytes leads to secondary oligodendrocyte loss and demyelination. Interestingly, spongiform demyelination is also evident in leukodystrophies associated with astrocyte dysfunction, such as Alexander disease and vanishing white matter disease (43). And, in a rat model of osmotic demyelination, astrocyte death occurs rapidly following correction of hyponatremia and outlines regions of future myelin loss. These observations suggest that the link between astrocyte damage and myelinolysis in NMO are independent of the mechanism of AQP4-IgG-mediated cytotoxicity. Possibilities include impaired potassium and cell volume regulation (44,45), excitotoxicity (46,47), altered astrocyte–oligodendroglial communication (48,49), release of inflammatory mediators (50), or deranged oligodendroglial metabolism (51) (Fig. 2).

Experimental models of NMO lesion formation are uniquely positioned to decipher the complex pathophysiology linking astrocyte loss and myelinolysis. In EAE (9,13), passive transfer (52) and ICI (12,13,27) NMO models, oligodendrocyte apoptosis and myelin vacuolization are apparent in regions of isolated astrocyte loss before inflammatory cell infiltration. Oligodendroglial cells are relatively preserved 1 hour after ICI of an AQP4 rAb and HC despite significant astrocyte depletion; 3 hours after injection, oligodendrocyte loss is readily apparent but myelin sheaths are spared (13). Within 12 hours of ICI, there is significant myelin edema and vacuolation (12), and by 18 hours, there is significant myelin fragmentation and vesiculated membrane profiles at the innermost layers of the sheath (27). Mechanistically, pathophysiology appears linked to increased intracellular calcium. Ionomycin treatment of acute brain slices results in myelin vesiculation and fragmentation identical to NMO lesions, and calcium chelation lessens myelin fragmentation in the NMO ICI model (27).

Recently, Herwerth et al (53) used intravital imaging to monitor the early phases of CNS lesion formation following the application of AQP4-IgG and HC to murine spinal cord *in situ*. Although oligodendrocytes remained relatively intact soon after the onset of astrocyte destruction, progressive axonal swellings were prominent (Fig. 2). Axonal swellings were not evident in the absence of the co-application of AQP-IgG and HC, and the number of

axonal swellings correlated with AQP4-Ig titer and the degree of astrocyte loss. Previous research has shown that axonal swellings demonstrate complex ultrastructural changes in the subaxolemmal cytoskeleton that may result from a variety of extrinsic stimuli (54). Potential pathogenic mechanisms are similar to those linking astrocyte destruction and myelinolysis: excitotoxicity (55), altered ion homeostasis (45), increased oxidative stress (56), and reduced axonal metabolic support (57,58). Experimental manipulation of neuronal signaling and metabolism in NMO animal models should help to clarify the relationship of progressive axonal swelling, oligodendrocyte apoptosis, and myelinolysis in subacute NMO lesions. The results may identify novel therapeutic avenues for the treatment of acute attacks.

Complement-Mediated Cytotoxicity: NMO Lesion Initiation and Propagation

Although noninflammatory mechanisms are likely to drive oligodendrocyte injury after astrocyte loss, NMO animal models consistently indicate that AQP4-IgG alone is insufficient to initiate demyelination. NMO lesions in the ICI model require the addition of HC (13,59–61) (Fig. 1). In the absence of HC, in the presence of complement inhibitors, or CDC-deficient AQP4 rAb, there is no evidence of astrocyte or oligodendrocyte loss. Similarly, in the rat where there is a robust endogenous complement system, CNS lesion formation by peripherally administered AQP4-IgG is abrogated by the administration of cobra venom factor, an inhibitor of complement function (52). Similarly, NMO lesion formation is greatly reduced after inhibiting antibody-dependent cell-mediated cytotoxicity (ADCC) using mutated AQP4 rAb, Fc γ RIII receptor-deficient mice, or a Fc γ receptor blocking antibody (61). Interestingly, CNS lesions initiated by ADCC cause astrocyte destruction but limited myelin loss (62). Therefore, astrocyte destruction by AQP4-IgG requires antibody effector function (CDC or ADCC), but only CDC is sufficient to initiate secondary demyelination.

CDC could be critical for demyelination in NMO lesions due to the combined production of anaphylatoxins (C3a, C4a, and C5a) and opsonins (C3b, C4b, and C1q) that recruit inflammatory cells (63), enhance polymorphonuclear-mediated ADCC (64), and facilitate phagocytosis (65) (Fig. 1). Anaphylatoxins are chemoattractants for polymorphonuclear cells (66). Indeed, experimental lesions initiated in the absence of complement show significantly reduced immune cell infiltration (62). After attracting granulocytes to the lesion site, C3a, C5a, and C1q promote further tissue injury by mediating eosinophil (67,68) and neutrophil degranulation (69) and free radical production (70). In NMO lesion models, inhibition of Fc receptor (FcR) binding or signaling significantly lessens tissue damage (62,68). Finally, opsonins (C3b and C4b) are important for phagocytic clearance of apoptotic cells (71,72). Macrophages are abundant in active demyelinating NMO lesions and constitute the major phagocytic population removing myelin debris (37,38,42). Opsonin production in NMO lesions may be critical for subsequent inflammatory demyelination by targeting apoptotic oligodendrocytes for phagocytosis (62).

Potential Noninflammatory Mechanisms of CNS Injury

In NMO, AQP4-IgG binding to CNS astrocytes has been reported to alter surface expression of AQP4 and diminish water channel function (Fig. 1). On the plasma membrane, AQP4 forms supramolecular assemblies, termed orthogonal arrays of particles (OAPs), that directly

impact AQP4-IgG binding (7). In tissue culture cells, AQP4-IgG causes internalization of surface AQP4 (46,73–75). Hinson et al (73) reported differential internalization of M1-AQP4 tetramers resulting in increased OAP size and potentially enhanced CDC. Other investigators, however, have failed to reproduce these findings (74–76). The effects of AQP4-IgG on surface AQP4 expressed by primary murine astrocytes and mixed glial cultures have been equally inconsistent (47,74,75,77,78). In mouse models, systemic or intracerebral administration of AQP-IgG failed to cause internalization of astrocyte AQP4 or tissue injury (10,12,74). However, in rats, chronic intrathecal infusion of large amounts of AQP4-IgG over 3 weeks resulted in local depletion of AQP4 in the adjacent spinal cord and reversible myelopathy (79). Interestingly, similar histopathology has been described in the cortex of cognitively impaired NMO patients (80) and in the vicinity of active NMO lesions (81).

The astrocyte glutamate transporter EAAT2 is complexed with AQP4 in astrocyte membranes and downregulated in AQP4-deficient mice (82). In human NMO spinal cord lesions, there is reduced EAAT2 expression in AQP4-deficient regions (77), suggesting that impaired glutamate reuptake and secondary excitotoxicity may contribute to CNS tissue injury. However, like observations regarding AQP4 internalization in transfected cells and primary cultures, there are conflicting reports on changes in EAAT2 expression and glutamate transport after exposure to AQP4-IgG (46,47,74,79). In mixed glial cultures, treatment with AQP-IgG in the absence of complement causes oligodendrocyte injury that is reduced with N-methyl-D-aspartate receptor antagonism (47). EAAT2 expression is also reduced following chronic intrathecal infusion of AQP4-IgG; however, the contribution of excitotoxicity to tissue injury in this model was not examined (79).

AQP4-IgG may also directly inhibit water channel function. In *Xenopus* oocytes expressing AQP4 water channels, AQP4-IgG was observed to delay cell lysis under hypotonic conditions (73). In contrast, neither AQP4-IgG nor AQP4-specific rAb was found to inhibit AQP4 water permeability in reconstituted M1-AQP4 proteoliposomes or M23-AQP4 plasma vesicles (75). The differential effect on oocyte water permeability is likely due to an artifact of experimental design as neither astrocyte nor myelin edema is noted in AQP4-deficient mice (83) or after administration of large amounts of intrathecal AQP4-IgG (12,79).

CENTRAL NERVOUS SYSTEM SPECIFICITY IN NEUROMYELITIS OPTICA

Although AQP4 is expressed in multiple tissues, NMO pathology is remarkably limited to the CNS. Transient serum elevation in creatine kinase is the most common observation of extra-CNS pathology (84,85); less frequently, muscle pathology with immune infiltrates, AQP4 loss, and complement deposition is evident (86–88).

CNS-specific injury in NMO is not determined by access to AQP4 in peripheral tissue. Serum AQP4-IgG or AQP4-specific rAb injected into rodents binds readily to AQP4 in kidney, stomach, lung, retina, and muscle in the periphery and the circumventricular organs and area postrema in the CNS (52,89). NMO pathology and complement deposition are not observed in peripheral tissues in the absence or presence of inflammation (10,52,89), indicating that CDC is not effectively activated in these tissues. Plasma membrane

complement regulatory proteins CD46, CD55, and CD59 are co-expressed with AQP4 in kidney, stomach, and skeletal muscle, whereas CD59 seems to be excluded from AQP4-positive astrocyte processes contacting endothelial cells (90). Indeed, intrathecal injection of NMO-IgG and HC in CD59 knockout mice produces longitudinally extensive spinal cord lesions and exacerbates ON in passive transfer models (91,92).

AQP4 OAPs are essential for AQP4-IgG-mediated CDC (76). Therefore, the abundance and size of AQP4 OAPs may contribute to the observed distribution of pathology in NMO. The relative expression of AQP4 mRNA and protein is higher in optic nerve and spinal cord than peripheral tissues (93), and optic nerve tissue demonstrates the highest ratio of large to small AQP4 supramolecular aggregates (93). CNS injury may be further polarized due to the cellular distribution of larger AQP4 supramolecular aggregates. Spinal cord white matter astrocyte processes display diffuse expression of AQP4 OAPs, whereas AQP4 OAP expression in grey matter astrocytes is polarized (94). Super resolution microscopy of spinal cord sections demonstrates large AQP4 clusters in both perivascular and parenchymal white matter but biased localization to perivascular grey matter (95). The diffuse expression of AQP4 OAPs in spinal cord and optic nerve white matter may provide an anatomic explanation for the longitudinal extension of optic nerve and spinal cord lesions in affected patients (14,28).

NOVEL APPROACHES TO THE TREATMENT OF ACUTE NEUROMYELITIS OPTICA LESIONS

Data from NMO experimental models provide an outline for the development of novel therapeutic approaches for the treatment of acute exacerbations. Both inflammatory and noninflammatory pathologic mechanisms may be addressed through various strategies. Small molecules and synthetic peptoid ligands that block AQP4-IgG binding have been identified in high-throughput screens (96–98). With suitable potency, half-life, and CNS penetration, these agents may offer a fundamental mechanism for limiting CNS lesion propagation during relapse. Alternatively, an AQP4-specific blocking antibody devoid of effector activity (aquaporumab) could be used to inhibit serum AQP4-IgG binding (60). As reviewed earlier, there is no definitive evidence for noninflammatory AQP4-IgG-mediated lesion pathology in experimental systems (10,12,61,99), and there is no evidence that AQP4-IgG directly inhibits AQP4 water channel function (75).

Both CDC and ADCC are necessary for complete lesion formation in the ICI animal model (61), and ADCC seems to play a critical role in facilitating macrophage-mediated demyelination at the outer rim of developing lesions (100). A C1-esterase inhibitor was recently shown to be safe in a small open-label treatment trial of acute NMO TM (101); however, high-dose therapy of acute lesions in an animal model was ineffective (102). In contrast, a C1q-targeted monoclonal antibody demonstrated effective inhibition of AQP4-IgG-mediated CDC in vitro, ex vivo, and in vivo (103). Treatment of acute relapses in NMOSD patients with complement inhibitors, however, may face unique therapeutic challenges due to limited CNS penetration, abundant target proteins, and intrathecal production of complement components.

Inhibition of eosinophil degranulation and neutrophil elastase with Silevestat (69,104) and cetirizine (68) has shown promise in animal models, and clinical trials with an alpha-1-antitrypsin inhibitor ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02087813): NCT02087813) and cetirizine ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02865018): NCT02865018) are currently underway. Since IVIg may interfere with ADCC through the upregulation of inhibitory FcR expression (105), administration of IVIg may offer another strategy for acute NMOSD relapses unresponsive to corticosteroids ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01845584): NCT01845584).

Recent histologic analysis of mitochondria in affected optic nerves from NMOSD patients suggest that disordered mitochondrial dynamics may contribute to retinal ganglion cell injury and loss (106). Lesioned optic nerve showed increased staining for transient receptor potential melastatin 4 (TRPM4) cation channels that may contribute to axonal injury. TRPM4 channels are directly inhibited by the antidiabetic drug glibenclamide, offering a novel avenue for mitigating axonal injury in ON (107).

In summary, multiple avenues of investigation in the laboratory have significantly advanced our understanding of NMO pathophysiology. The result is a framework for understanding immunologic and nonimmunologic mechanisms that lead from a targeted antibody response against astrocytes to demyelination and neuronal injury. Consequently, the therapeutics pipeline is rich with agents that target a wide range of pathways. Many offer significant appeal for acute therapy by selectively targeting pathways that propagate and amplify CNS lesions. Moving these agents from the bench to the bedside offers the opportunity to identify safe and effective therapies that limit CNS injury and preserve visual function.

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FIG. 1.

Inflammatory and noninflammatory mechanisms contributing to astrocyte injury in neuromyelitis optica. Inflammatory mechanisms include complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), opsonization, and complement-induced degranulation. Potential noninflammatory mechanisms contributing to injury include aquaporin-4 (AQP4) and glutamate transporter (EAAT2) internalization and direct inhibition of AQP4-mediated water transport. AP, alternative pathway; C2aC4bC3b, C5 convertase; C4bC2a, C3 convertase; CP, classical pathway; MAC, membrane attack complex; NK, natural killer.

FIG. 2.

Myelinolysis and progressive axonal swelling following astrocyte injury in neuromyelitis optica lesions. Astrocyte destruction mediated by AQP4-IgG results in early myelinolysis and progressive axonal swelling. The potential mechanisms driving these pathologies include inflammatory, metabolic, ionic, and excitotoxic mechanisms. Progressive axonal swellings identified in intravital microscopy may represent periaxonal swelling or myelin injury (splitting or focal bulging). Adapted from (27). Ca^{2+} , calcium.