

# T cells targeting neuromyelitis optica autoantigen aquaporin-4 cause paralysis and visual system injury

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**Aquaporin-4 (AQP4)-specific antibodies are instrumental in promoting central nervous system (CNS) tissue injury in neuromyelitis optica (NMO), yet evidence indicates that AQP4-specific T cells also have a pivotal role in NMO pathogenesis. Although considerable effort has been devoted to creation of animal models to study how AQP4-specific T cells and antibodies may cooperate in development of both clinical and histologic opticospinal inflammatory disease, the initial attempts were unsuccessful. Recently, it was discovered that T cells from AQP4-deficient (AQP4<sup>-/-</sup>) mice recognize distinct AQP4 epitopes that were not identified previously in wild-type (WT) mice, and that donor Th17 cells from AQP4<sup>-/-</sup> mice that target those novel epitopes could cause paralysis and visual system injury associated with opticospinal inflammation in WT recipient mice. These observations indicate that the pathogenic AQP4-specific T cell repertoire is normally controlled by negative selection. Here, we describe the advances leading to development of an animal model for aquaporin-targeted CNS autoimmunity (ATCA). This new model provides a foundation to investigate immune mechanisms that may participate in NMO pathogenesis. It should also permit preclinical testing of agents considered for treatment of NMO.**

neuromyelitis optica | aquaporin-4 | T cells | experimental neuromyelitis optica | experimental autoimmune encephalomyelitis

Neuromyelitis optica (NMO) is a CNS autoimmune inflammatory demyelinating disease that can cause severe, disabling paralysis and visual loss [1]. Unlike multiple sclerosis (MS), a more common CNS inflammatory disease that is considered to primarily target myelin proteins produced by oligodendrocytes, the target in NMO is aquaporin-4 (AQP4), a water channel expressed abundantly on astrocyte end-foot membranes in areas contacting the blood-brain barrier [2]. Pathogenic antibodies to AQP4 are found in the serum of approximately 75% of NMO patients [3]. NMO is therefore considered a humoral autoimmune disease. However, there is increasing evidence linking T cells to the pathophysiology of NMO. AQP4-specific antibodies are IgG1, a T-cell dependent Ig subclass [4] and their entry to the CNS requires inflammation [5, 6]. NMO patients have a higher frequency of AQP4-reactive T cells than healthy controls, and those T cells produce IL-17, providing evidence for a role of Th17 cells in NMO pathogenesis [7]. Yet, the precise contribution of T cells to opticospinal inflammation and neuronal damage in NMO has not been clarified. Animal models, which may elucidate how AQP4-specific T cells and antibodies cooperate in CNS autoimmunity are sorely needed.

Although multiple AQP4 T cell epitopes have been identified in WT mice and rats [8-10], development of a model with clinical and histologic manifestations of opticospinal autoimmune disease that targets AQP4 has proven challenging. Immunization with those identified peptide determinants or transfer of donor T cells

targeting those epitopes did not induce clinically evident AQP4-targeted experimental NMO (“ENMO”) [9-11]. Zeka et al. did detect inner retinal T cell infiltration with permanent retinal ganglion cell (RGC) injury in Lewis rats after transfer of AQP4 p268-285-specific T cells, with or without injection of NMO-IgG, but did not observe significant paralysis [12].

In recent work, Sagan et al. studied AQP4-specific T cell responses in both AQP4-deficient (AQP4<sup>-/-</sup>) and WT mice [13]. Robust proliferation was observed following immunization of AQP4<sup>-/-</sup> mice with AQP4 peptide (p) 135–153 or p201–220, the two AQP4 peptides predicted to bind MHC II (I-A<sup>b</sup>) with highest affinity. In contrast, these two AQP4 peptides elicited much weaker T cell responses in WT mice. The T cell receptor (TCR) repertoires used for recognition of AQP4 p135-153 and p201-220 in AQP4<sup>-/-</sup> mice were also unique. Collectively, these findings indicate that development and expansion of AQP4-specific T cells is regulated by thymic negative selection. Donor AQP4 p135-153-specific or p201–220-specific T cells from AQP4<sup>-/-</sup> mice caused acute paralysis that was accompanied by opticospinal inflammation in WT and B-cell-deficient mice. In comparison to Th1 cells, Th17-polarized AQP4-specific cells caused more severe clinical and histologic disease than AQP4-specific Th1 cells, providing further support for a Th17-mediated etiology in AQP4-targeted CNS autoimmunity (ATCA). Fluorescent-labeled AQP4-specific T cells were identified in infiltrates within the spinal cord and optic nerves, demonstrating that those pathogenic T cells had indeed penetrated the CNS. Optical coherence tomography (OCT) was used to monitor changes in the thickness of the inner retinal layers and revealed dynamic changes after induction of disease. Interestingly, although AQP4 is expressed in other organs (e.g., kidney and muscle) [14], AQP4-targeted T cell-mediated inflammation was detected in the CNS only.

Whereas AQP4 is the principal autoantigen in NMO, myelin oligodendrocyte glycoprotein (MOG), which itself is highly expressed on the surface of the outer lamellae of CNS myelin in the optic nerves and spinal cord [15], is a candidate autoantigen in multiple sclerosis (MS) [16-18]. Of interest, a subset of AQP4-IgG seronegative patients that have been diagnosed with NMO produce anti-MOG IgG [19]. While AQP4- and MOG-specific antibodies target different cell components of the CNS, relapsing optic neuritis is frequent in both groups of patients [20].

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In their report, Sagan et al. studied the differential features of MOG- and AQP4-targeted T cell-mediated CNS autoimmunity. They compared the disease course, retinal dynamics, histological and immunohistochemical findings in WT mice receiving either Th17-polarized AQP4 p201-220-specific or MOG p35-55-specific T cells. MOG-specific Th17 cells induced classic EAE, with persistent paralysis associated with axonal loss in the spinal cord as well as initial inner retinal swelling that led to atrophy and reduction of retinal ganglion cells (RGCs). In contrast, both paralysis and retinal swelling induced by AQP4-specific Th17 cells were self-limited and there was no loss of spinal cord axons or RGCs. These findings highlighted distinct roles for T cells targeting cell surface astrocyte- and oligodendrocyte-specific proteins in CNS autoimmunity, which should provide insight regarding their respective roles in NMO and MS.

It is recognized that AQP4-specific IgG1 can have a key role in the effector stage of NMO. In this regard, Felix et al. [21] found that intravitreal delivery of anti-AQP4 IgG reduced AQP4 expression and increased glial fibrillary acidic protein with retinal inflammation and loss of ganglion cells in the retinas of adult rats and, surprisingly, this primary retinal pathology was complement-independent. After Sagan et al. [13] first demonstrated that AQP4 contains two pathogenic T cell epitopes, p135-153 and p201-220, in mice, Vogel et al. [22] reported that T cell- and B cell-deficient (Rag1<sup>-/-</sup>) mice reconstituted with naive peripheral CD4<sup>+</sup> T cells from AQP4<sup>-/-</sup> mice were susceptible to induction of CNS autoimmunity by immunization with AQP4 p201-220. They also identified an NMO lesion-like pattern only upon concomitant administration of anti-AQP4 antibodies.

It took more than five years from the discovery of AQP4-specific T cell epitopes in mice [8] before investigators first succeeded in creating a model of clinical and histologically evident ATCA [13, 22, 23]. This advance was made by the discovery that pathogenic AQP4 T cell epitopes are concealed by the normal immune system. T cells that recognize the unique pathogenic AQP4 T cell determinants, identified by studying AQP4<sup>-/-</sup> mice, utilize both unique T cell and TCR repertoires, findings that indicate AQP4-specific T cells are normally sensitive to mechanisms of central tolerance. In future investigations, it will be important to examine whether specific gene products known to control thymic negative selection shape the AQP4-specific T cell repertoire in ATCA. One can speculate that a defect(s) in thymic negative selection might also participate in NMO pathogenesis. It is clear that the identification of multiple pathogenic AQP4 T cell epitopes should accelerate research to understand how pathogenic AQP4-specific T cells develop in NMO and how they may direct differentiation of AQP4-specific B cells and antibody-secreting plasma cells.

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