Anti-aquaporin-4 antibodies in Devic's neuromyelitis optica: therapeutic implications

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***Abstract*:** Devic's neuromyelitis optica (DNMO) is a demyelinating and inflammatory disease of the central nervous system (CNS) essentially restricted to the spinal cord and the optic nerves. It is a rare disorder with a prevalence estimated at less than 1/100,000 in Western countries. Since the first description by Eugène Devic in 1894, the relationship between DNMO and multiple sclerosis (MS) has been controversial. Recent clinical, epidemiological, pathological and immunological data demonstrate that MS and DNMO are distinct entities. This distinction between DNMO and MS is crucial, as prognosis and treatment are indeed different. DNMO is now considered to be an autoimmune, antibody-mediated disease especially since the identification of a specific serum autoantibody, named NMO-IgG and directed against the main water channel of the CNS, aquaporin-4 (AQP4). The assessment of AQP4 antibodies (Abs) has initially been proposed to differentiate DNMO and MS. It has also enlarged the clinical spectrum of DNMO and proved to be helpful in predicting relapses and conversion to DNMO after a first episode of longitudinally extensive transverse myelitis or isolated optic neuritis. Lastly, the discovery of the pathogenic role of AQP4 Abs in DNMO leads to a better understanding of detailed DNMO immunopathology and the elaboration of relevant novel treatment strategies specific to DNMO. In this review, we summarize the present and future therapeutic implications generated by the discovery of the various pathogenic mechanisms of AQP4 Abs in DNMO pathophysiology.

***Keywords*:** aquaporin-4, autoantibody, myelitis, neuromyelitis optica, treatment

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Introduction

Devic's neuromyelitis optica (DNMO) is a demyelinating and inflammatory disease of the central nervous system (CNS) essentially restricted to the spinal cord and the optic nerves. It is a rare disorder, with a prevalence estimated at less than 1/100,000 in Western countries. Since the first description by Eugène Devic in 1894 [Devic, 1894], the relationship between DNMO and multiple sclerosis (MS) has been controversial. However, recent clinical, epidemiological, pathological and immunological data have demonstrated that MS and DNMO are distinct entities [Wingerchuk *et al.* 2007a, 1999; Luchinetti *et al.* 2002; O'Riordan *et al.* 1996]. This distinction is crucial, as prognosis and treatment are indeed different. DNMO is now considered to be an autoimmune, antibody-mediated disease especially since the identification of a specific serum autoantibody, named NMO-IgG and directed against the main water channel of the CNS, aquaporin-4 (AQP4) [Lennon *et al.* 2005, 2004]. The identification of AQP4 antibodies (Abs) as a specific marker for DNMO spectrum disorders has profoundly improved our understanding of DNMO. In this review, we summarize the present and future therapeutic consequences generated by the discovery of the various pathogenic mechanisms of AQP4 Abs in DNMO.

Clinical features and prognosis

Clinically, DNMO is characterized by the association of unilateral or bilateral optic neuritis and acute transverse myelitis. Although a monophasic course of acute transverse myelitis simultaneously associated with optic neuritis is classical [Gault, 1895; Devic, 1894], more than 90% of the patients experience a relapsing–remitting course and possibly a long time interval between

neurological episodes. Optic neuritis in DNMO is more severe and recovery is less complete compared with attacks of optic neuritis in the context of MS. Spinal cord relapses typically present as a complete transverse myelitis with severe, symmetric paresis below the lesion, sensory loss below the lesion and severe sphincter disturbances. Prominent dysesthetic, radicular pain and even Lhermitte's symptom are common. Hiccup, intractable nausea, or respiratory failure may also occur as a result of the involvement of the medulla oblongata [Takahashi *et al.* 2008; Wingerchuk *et al.* 2007a]. Prognosis is usually poor: after 5 years of natural history, i.e. before the widespread use of immunosuppressive treatments, half of the patients lost their vision in one eye or were unable to walk without aid whereas the estimated survival rate was 68% [Wingerchuk *et al.* 1999]. By contrast with MS, disability seems to be acquired mainly, if not exclusively, as a consequence of relapses and there is usually no progressive phase in DNMO [Wingerchuk *et al.* 2007b]. It is expected that a better management of the disease with an earlier and more accurate diagnosis, an earlier initiation of the treatment, and the selection of relevant treatments will result in improvements in the course and the prognosis of DNMO. A recent French multicentre study of 125 patients tends to confirm this expectation. In this observational study most patients were under immunosuppressive therapy and the median time from onset to Expanded Disability Status Scale (EDSS) 6 was delayed to 10 years [Collongues *et al.* 2010].

Pathophysiology

Classically in DNMO lesions, inflammatory infiltrates are associated with cavitation, necrosis and acute axonal pathology in both grey and white matter of the spinal cord and optic nerves [Mandler *et al.* 1998].

Several lines of evidence support a prevailing role of humoral immunity in DNMO pathogenesis. Lesion pathology is characterized by perivascular deposits of immunoglobulins (mainly IgM) and complement C9 neoantigen. Immunoglobulin and complement components are found in a specific vasculocentric rim and rosette pattern [Luchinetti *et al.* 2002]. Circulating autoantibodies are frequently found in DNMO and their presence may also reflect a more widespread B-cell response [Pittock *et al.* 2008]. These autoantibodies might cause damage directly through the recognition of epitopes on normal cells, or indirectly through the formation of immune complexes that deposit in normal tissue and activate the complement cascade.

The discovery of NMO-IgG and the enlargement of the DNMO spectrum

NMO-IgG is a highly specific autoantibody found in DNMO. It was initially proposed to differentiate DNMO and MS, with a sensitivity and specificity of 73% and 91% respectively [Lennon *et al.* 2004]. Several studies from different countries have confirmed these data [Marignier *et al.* 2008; Jarius *et al.* 2007; Zuliani *et al.* 2006]. Identification of this serum autoantibody has enlarged the clinical spectrum of DNMO [Wingerchuk *et al.* 2007, 2006], including clinically monofocal cases previously termed idiopathic single or recurrent longitudinally extensive transverse myelitis, recurrent or simultaneous bilateral optic neuritis [Petzold *et al.* 2010] and Japanese opticospinal MS. The spectrum has also been enlarged to DNMO cases with brain MRI lesions, symptomatic or not [Pittock *et al.* 2006a]. Most of them were nonspecific, but 10% had MS-like lesions and a very small number of patients had diencephalic, brainstem or cerebral lesions considered as atypical for MS but specific for DNMO [Carlander *et al.* 2008; Pittock *et al.* 2006b] or associated with systemic autoimmune diseases [Pittock *et al.* 2008]. By contrast, only 20% of the sera from monophasic patients were positive for NMO-IgG and titres were generally lower suggesting that monophasic DNMO and relapsing DNMO may not be the same entity [Waters *et al.* 2008].

Aquaporin-4

The antigenic target for NMO-IgGs has been identified as AQP4 [Lennon *et al.* 2005] and NMO-IgGs are now considered as anti-AQP4 Abs. Indeed, new tests using transfected cells with high amounts of human AQP4 have been developed, but results are controversial and the ideal detection method remains to be elucidated [Fazio *et al.* 2009; McKeon *et al.* 2009; Waters *et al.* 2008; Takahashi *et al.* 2007].

In 1992 Peter Agre and colleagues identified the first member of the aquaporin family [Preston *et al.* 1992]. To date, this family has 11 members in mammals. Aquaporins have been found to facilitate transmembrane transport of water in a number of organs. Aquaporin-4 is the main water channel of the CNS and is expressed on the plasma membrane of astrocytes,

but not oligodendrocytes. It is concentrated in domains that face the basal membrane of endothelial cells [Amiry-Moghaddam *et al.* 2003]. Aquaporin-4 is expressed as two major isoforms of 32 kDa (AQP4-M1) and 30 kDa (AQP4-M23) [Lu *et al.* 1996] polarized at the astrocyte plasma membrane and organized as tetramers where it is ultrastructurally arranged in the form of the so-called orthogonal arrays of particles (OAPs) [Yang *et al.* 1996]. Interestingly it has been recently shown that the ratio of M1 : M23 mRNA is highest in the optic nerve and spinal cord, the target tissue of DNMO, followed by brainstem, then the cerebral and cerebellar cortices [Saini *et al.* 2010]. It is generally agreed that AQP4 plays a role in brain water control [Papadopoulos *et al.* 2004]. However, recent works have indicated that AQP4 functions in the CNS are broader than expected [Verkman *et al.* 2006]. In astrocytes, AQP4 has been functionally associated with the glutamate transporter type 1 (GLT1), and the inward rectifying K^+ channel Kir 4.1. It is also part of the dystrophin protein complex (DGC complex), a macromolecular complex which plays an important role in K^+ buffering, extracellular space expansion, neuronal transmission and excitability [Zeng *et al.* 2007; Verkman, 2005; Binder *et al.* 2004].

Aquaporin-4 in DNMO pathophysiology

Antibodies can have a wide range of effector functions, either acting as receptor agonist or antagonist, or activating complement cascades and Fc receptor, or altering antigen density in the target cells [Diamond *et al.* 2009]. As for AQP4 Abs, several recent findings support their direct pathogenic role in DNMO. Clinically, AQP4 Ab titres are associated with extension of spinal cord and brain lesions [Takahashi *et al.* 2007] and some patients present brain MRI lesions in sites of high AQP4 expression [Pittock *et al.* 2006b]. In pathological human material, deposition of immunoglobulins and complement is preferentially located in areas of high AQP4 expression and AQP4 is lost selectively in active DNMO lesions [Misu *et al.* 2007; Roemer *et al.* 2007]. In animal models, intraperitoneal injection of human sera containing NMO-IgG induces NMO-like lesions in a context of T-cell-mediated brain inflammation [Bradl *et al.* 2009]. More recently, the same NMO-like lesions were induced by intracerebral co-injection of human sera containing NMO-IgG and human complement, but not with sera containing NMO-IgG alone [Saadoun *et al.* 2010]. *In vitro* experiments showed that the binding of NMO-IgG to AQP4 expressed at the astrocyte membrane can initiate complement activation and complement-dependent cytotoxicity [Kinoshita *et al.* 2009; Sabater *et al.* 2009; Vincent *et al.* 2008; Hinson *et al.* 2007]. In the absence of complement, NMO-IgG binding induces a reversible internalization of the AQP4-IgG complex and a loss of expression of the glutamate transporter type 1 GLT1/EAAT2 [Hinson *et al.* 2008]. Finally, we recently demonstrated in the laboratory in *in vitro* and *ex vivo* complement-free models that NMO-IgG profoundly damages oligodendrocytes through an excitotoxic mechanism resulting from glutamate homeostasis disruption in astrocytes [Marignier *et al.* 2010]. Thus, IgGs of patients with AQP4 Abs can severely impair astrocyte survival, metabolism and function.

Current treatment

Devic's disease is a rare disorder for which no therapeutic controlled trials, neither for acute relapses nor for prevention, have been conducted. Therapeutic recommendations are based only on case series and expert opinions.

Relapse therapy

Taking into account the possible early and severe attack-related disability that occurs in DNMO, relapse therapy is typically considered as an emergency, by contrast with MS. The accumulative evidence of direct deleterious action of the serum AQP4 Abs in DNMO and the fact that a possible reversible homeostatic dysfunction induced by these autoantibodies may precede irreversible tissue destruction [Hinson *et al.* 2007] are another strong argument for treating relapse as early as possible.

Classically, high doses of intravenous methylprednisolone (IVMP) are the first line of treatment in patients presenting with a DNMO attack. Corticosteroids reduce the inflammatory response via multiple mechanisms, including downregulation of B lymphocytes [Franchimont, 2004]. Plasma exchanges (two to five courses) are also effective in the management of DNMO attack but are usually used only in the case of failure of IVMP [Watanabe *et al.* 2007; Keegan *et al.* 2002; Weinshenker *et al.* 1999]. However, the presence of a humoral toxic agent could argue for the superiority of plasma exchanges relatively to IVMP, as this is the case for other antibody-driven neuro-inflammatory disorders such as

myasthenia gravis or limbic encephalitis [Vincent, 2010]. Interestingly, a recent work suggests that plasma exchanges could be more efficient than IVMP to prevent relapse-related disability [Bonnan *et al.* 2009].

More anecdotally, in three patients, intravenous polyvalent immunoglobulins (IVIGs) have been reported to have a possible effect as relapse therapy [Okada *et al.* 2007; Bakker and Metz, 2004]. A positive effect of lymphocytapheresis as rescue therapy in DNMO has been described in a small number of cases [Moreh *et al.* 2008; Nozaki *et al.* 2006] suggesting that cellular immunity may be critical. Indeed, lymphocytapheresis results essentially in reducing clonally expanding pathogenic T cells without influencing circulating antibodies.

Prevention therapy

Usually, relapse prevention in DNMO is based on early and maintenance immunosuppressive treatments [Wingerchuk *et al.* 2007a, 1999]. Considering the antibody-driven hypothesis in DNMO, treatment should target B cells. Mycophenolate mofetil and azathioprine, that have demonstrated cytostatic effects on B and T cells, and mitoxantrone, which is known to target predominantly B cells and macrophages, have shown their efficacy in small cohorts of DNMO patients [Jacob *et al.* 2008; Weinstock-Guttman *et al.* 2006; Mandler *et al.* 1998].

By contrast, immunomodulatory beta-interferons (beta-IFNs), a classical first-line treatment for MS, are usually inefficient or even deleterious in DNMO [Papeix *et al.* 2007; Warabi *et al.* 2007]. In fact, beta-IFN is an inductor of the B-cell activating factor of the TNF family (BAFF) [Krumbholz *et al.* 2008], a B-cell survival factor that enhances antibody production [Mackay *et al.* 2007]. BAFF could be involved in DNMO pathophysiology as a recent work has demonstrated an upregulation of BAFF in the serum and CSF of DNMO patients as compared with MS patients and controls [Okada *et al.* 2010]. Although glatiramer acetate has been associated with stabilization of the disease in two case reports, it can currently not be considered as a useful drug in DNMO prevention [Sellner *et al.* 2010].

Finally, B-cell depletion therapy, particularly using anti-CD20 treatment, has provided a proof of concept that targeting B cells may result in clinical improvements in autoimmune diseases [Dörner *et al.* 2006]. Monoclonal antibodies directed against B-cell surface markers bind to the target antigen and kill the cell by initiating a combination of apoptosis, complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cellular cytotoxicity (ADCC) [Cragg and Glennie, 2004]. Rituximab (RTX), a chimeric monoclonal antibody that specifically targets CD20, is currently the more promising approach for DNMO therapy. CD20 is a human B lymphocyte-specific antigen that is expressed on immature, mature and memory B cells from blood and lymphoid organs but not on antibody-producing plasma cells. However, B cells are essential for pathogenesis of recurrent attacks in NMO probably not only through autoantibody production, but also acting as antigen presentation cells. The beneficial effects on DNMO have been emphasized recently in a monocentric open-label study [Cree *et al.* 2005] and a multicentric, retrospective study [Jacob *et al.* 2008]. However, one has to keep in mind that some patients remain refractory to RTX [Cassinotto *et al.* 2008; Capobianco *et al.* 2007], despite complete B-cell depletion, characterized by undetectable blood $CD19^{+}$ cells. One explanation could be the possible existence of long-lived plasma cell clones that still produce AQP4 Abs and that are not targeted by RTX as they do not express CD20. Another explanation is that other types of immune cells (T cells), not targeted by RTX, are possibly involved in DNMO pathophysiology. Finally, it has been recently observed that RTX infusion could induce a transient increase of AQP4 Abs titres by upregulating the BAFF, but without associated clinical attack [Nakashima *et al.* 2009].

AQP4 antibodies in the current management of DNMO

After a first episode of DNMO

Diagnostic value. In case of initial clinical involvement of both the optic nerve and the spinal cord, AQP4 Abs are of limited interest for diagnosis. Whatever the serological status for AQP4 Abs, the diagnosis is definite DNMO and a long-term immunosuppressive treatment is required. Indeed, one has to keep in mind that sensitivity of the detection of AQP4 Abs is around 60–70% [Waters *et al.* 2008]. Anyway, in this particular clinical presentation,

AQP4 Abs are still helpful in atypical cases with large brain involvement or in association with other autoimmune diseases.

AQP4 Abs are of greatest value after a clinically monofocal episode, i.e. optic neuritis, transverse myelitis (more rarely intractable hiccup or nausea or sleep disturbances). Considering the very high specificity of AQP4 Abs, its association with such clinical presentation is highly suggestive of DNMO spectrum disorder. It is now well accepted that these syndromes should be considered as inaugural monofocal forms of DNMO as they share the same pathophysiology. Recent findings of spinal cord biopsy analysis from one patient with acute transverse myelitis and AQP4 Abs demonstrated extremely active demyelination of plaques, thickening of hyalinized blood vessels, extensive loss of AQP4 in lesion, and diffuse infiltration by macrophages, identical to those of autopsy from definite DNMO [Yanagawa *et al.* 2009].

Prognostic value. AQP4 Abs are useful to predict relapses or conversion to DNMO after a first episode of longitudinally extensive transverse myelitis or optic neuritis. Indeed 62.5% of NMO-IgG positive patients suffering from longitudinally extensive transverse myelitis experienced a second episode within a year (transverse myelitis or optic neuritis) whereas no NMO-IgG seronegative patients relapsed [Weinshenker *et al.* 2006]. The same results were observed for recurrent isolated optic neuritis with a significant higher risk of conversion to definite DNMO in the NMO-IgG seropositive group [De Sèze *et al.* 2008; Matiello *et al.* 2008].

Thus, in case of an inaugural bifocal episode or after a monofocal episode seropositive for AQP4 Abs, a maintenance therapy is required to prevent further potentially devastating episodes. While only very few data are available in the literature concerning treatment of monofocal DNMO, no clear differences appeared compared with definite DNMO in terms of treatment response or prognosis. Early diagnosis, made possible by AQP4 Abs detection, should lead to optimized management and may prevent further relapse and poor outcome in DNMO.

Follow up: monitoring

Some recent data have emphasized a possible link between NMO-IgG/AQP4 Abs titres, clinical activity and response to treatment [Weinstock-Guttman *et al.* 2008; Takahashi *et al.* 2007]. Longitudinal follow up of a small cohort of patients has shown that low titres of AQP4 Abs were associated with a quiescent phase of the disease and that immunosuppressive treatment resulted in a marked reduction in AQP4 Abs level [Jarius *et al.* 2008]. These results could be of particular interest for the management of specific therapy where markers for the timing of reinfusion are lacking. Presumably, the intensity and the duration of the immunosuppressive maintenance therapy might be adapted according to the evolution of the antibodies against AQP4, knowing that effective therapy can transform the AQP4 Abs into a negative status [Weinstock-Guttman *et al.* 2008]. However, these data are preliminary and longitudinal follow-up studies on large populations are needed to clearly answer this question.

AQP4 antibodies and future therapeutic approaches

New molecules and techniques

Plasmapheresis. In the near future, specific depletion of anti-AQP-4 Abs based on techniques that associate the immunoadsorption column and apheresis system could be used during attack of DNMO. Indeed, in myasthenia gravis, a protopathic antibody-mediated disease, a specific therapeutic apheresis of anti-acetylcholine receptors (AChR) autoantibodies from patients' sera using the immobilized subunits of AChR as immunoadsorbents was recently successfully achieved [Zisimopoulou *et al.* 2008]. The ability of the anti-AChR antibodies isolated by this technique, but not that of the depleted sera, to induce disease, demonstrated the efficacy of the method.

Anti-B-cell therapy. In addition to RTX, there are several other potential candidates useful in B-cell depletion therapy. First, ocrelizumab, the humanized version of RTX, is under development in various autoimmune disorders [Genovese *et al.* 2008]. Second, new monoclonal antibodies that specifically target CD19 and CD22 and modulate B-cell populations are now becoming available. CD19 is a cell surface response regulator that establishes signalling thresholds critical for B-cell development and activation [Sato *et al.* 1996]. It is expressed from the early stage of B-cell maturation and is present on memory B cells and some plasma

cells, which is in contrast to CD20 [Stashenko *et al.* 1980]. CD22 is a lectin that is expressed by all mature B lineage cells but surface expression ceases when mature B cells differentiate into plasma cells. CD22 modulates CD19 signal transduction, providing essential survival signals [Tedder *et al.* 2005]. Epratuzumab, which blocks CD22 thereby causing mature B-cell depletion, is currently under development. Third, protein fusion that targets BAFF or APRIL (a proliferation inducing ligand), two members of the TNF family crucial for B-cell survival [Dalakas, 2008] are under development.

New therapeutic approaches

Considering that AQP4 Abs plays a direct and early pathogenic role in DNMO lesion formation, future approaches should focus on treatment that could directly target AQP4 Abs and prevent the deleterious cascade following its interaction with membrane AQP4.

Blockade of antigen-antibody interaction. In the CNS, AQP4 is ultrastructurally arranged into homotetramers and heterotetramers that form the OAPs at the astrocyte endfeet close to the blood–brain barrier. OAPs are made of both M1 and M23 isoforms and this ratio is important in determining the size of the OAPs [Rossi *et al.* 2010]. Considering that AQP4-Abs may not be directed against the AQP4 protein itself but against the OAPs [Nicchia *et al.* 2009], it had been proposed to modulate the AQP4-M23 isoform expression to prevent the aggregation of AQP4 tetramers into OAPs and thus blocking the interaction with serum AQP4 Abs [Warth, 2009]. Potential targets which may be therapeutically modulated to avoid OAP formation already exist as it has been demonstrated that OAP formation is inhibited by palmitoylation of N-terminal cysteine residues [Suzuki *et al.* 2008].

Modulation of the dystrophin protein complex associated with AQP4 at the astrocyte endfeet may also prevent interaction with membrane AQP4. For instance, in the brain of alpha-Syntrophin (−/−) mice, the total level of AQP4 expression appears normal but the polarized subcellular localization is reversed. High-resolution immunogold analyses revealed that AQP4 expression is markedly reduced in astrocyte endfeet membranes adjacent to blood vessels, but is present at higher than normal levels in membranes facing neuropil [Neely *et al.* 2001].

Blockade of complement activation. Release of activated complement components is thought to play an important role in DNMO pathophysiology [Kinoshita *et al.* 2009; Sabater *et al.* 2009; Hinson *et al.* 2007; Roemer *et al.* 2007; Lucchinetti *et al.* 2002]. Of note, AQP4 Abs are mainly IgG1, one of the most potent antibody isotypes for complement activation [Waters *et al.* 2008].

The anticomplement approach is a potentially attractive therapeutic strategy. In an animal model of DNMO the toxic effect of human AQP4 Abs was partially blocked by the co-injection of complement C1 inhibitor [Saadoun *et al.* 2010]. Eculizumab, a humanized monoclonal antibody that blocks the cleavage of C5 and halts the process of complement-mediated cell destruction, has demonstrated promising results in a model of antibody-mediated peripheral neuropathy [Halstead *et al.* 2008]. A phase I/II open-label study of the effects of eculizumab in DNMO is currently recruiting patients (see http://clinicaltrials.gov/ct2/show/NCT00904826). Another promising anticomplement therapy is TT30, a fusion protein that leads to both local control of inflammation as well as a substantial decrease in the autoimmune response *in vivo*. Finally C1 inhibitor may be a practical point for future therapeutic intervention in NMO patients in order to prevent complement cascade. However, one has to keep in mind the dual role of complement during neuro-inflammation as C5b-9 was also shown to protect oligodendrocytes from apoptosis both *in vitro* and *in vivo* [Rus *et al.* 2006].

Antiglutamate therapy. Downexpression of both AQP4 and the glial glutamate transporter GLT1/EAAT2 on the plasma membrane of astrocytes has been observed following exposure of isolated astrocytes to NMO-IgG in the absence of complement [Marignier *et al.* 2010; Hinson *et al.* 2008]. White matter injury observed in DNMO lesions could be related to changes in astrocyte homeostasis and elevated extracellular glutamate level as a consequence. Astrocyte can release a variety of gliotransmitters, including glutamate [Parpura and Zorec, 2009; Parpura *et al.* 1994] and oligodendrocytes are highly vulnerable to this main excitotoxic transmitter in the spinal cord and optic nerve [Marignier *et al.* 2010; Xu *et al.* 2008; Matute, 2006; Matute *et al.* 1997]. N-methyl-D-aspartate receptors (NMDAR) are glutamate receptors that might play a major role

in such oligodendrocyte injury. These receptors are functionally expressed in the myelinating processes of oligodendrocytes [Karadottir *et al.* 2005]. Blockade of NMDAR reduced damage to white matter in a model of multiple sclerosis [Basso *et al.* 2008; Wallstrom *et al.* 1996] and overactivation leads to disintegration of oligodendroglial processes in the optic nerve that could be prevented by specific blockade [Salter and Fern, 2005]. Treatment leading to the prevention of extracellular glutamate accumulation should prevent excitotoxic oligodendrocyte loss and demyelination. Memantine, a specific antagonist to NMDAR, or riluzole, another antagonist to NMDAR that also inhibits the release of glutamate by interfering with sodium (Na^+) channels, could be proposed as an original and effective therapeutic strategy for protecting myelin and axonal loss during DNMO attacks.

Blockade of osmotically induced demyelination. Autoimmune loss of AQP4 water channels in astrocyte endfeet of DNMO patients induced by AQP4 Abs may lead to a disruption of an essential water exit pathway for the panglial syncitium. This syncitium is a vast network of interconnected oligodendrocytes and astrocytes that maintains ionic conditions. Following axonal saltatory conduction, the siphoning of large volumes of intracellular osmotic water that accompanies K^+ is required for myelin integrity. The pathological disruption of this water exit pathway induced by NMO-IgG might lead to osmotic load of myelin, resulting ultimately in oligodendrocyte loss and demyelination as proposed by Rash [Rash, 2009]. Disruptions of myelin should be particularly pronounced in active white matter tracts, including the optic nerve, the axons of the constantly activated descending respiratory motor tracts of the spinal cord, the corpus callosum, all regions targeted by DNMO lesions [Pittock *et al.* 2006b].

The initial swelling changes might be partially reversed, before necrosis of the majority of oligodendrocytes occurs, using therapies that reduce Na^+ influx, and hence reduce K^+ efflux during normal saltatory conduction.

DNMO patients seronegative for anti-aquaporin 4 antibodies

Despite a clinically well-established diagnosis of DNMO, 30–40% of patients remain seronegative for AQP4 Abs. Explanations for the seronegative rate include technical limits of current assays. Indeed, some seronegative patients may have Ab titres below the threshold of detection of the current assays, although the use of human AQP4 expressed at a high concentration in cell lines, is likely to be optimal for detection of the antibodies. Second, active and long-run immunodepression induced by therapy, might decrease the serum level of autoantibodies and, thus, result in a negative test. Third, it is probable that, like in other autoimmune diseases, there may be more than one target antigen. A study using protein microarray identified three new autoantibodies possibly implicated in NMO pathogenesis [Lalive *et al.* 2006]. The future recognized target antigen could be helpful for monitoring new therapies. Finally, it cannot be ruled out that in certain patients, cell-mediated autoimmunity may have a predominant role in certain cases, as already mentioned.

However, it has been demonstrated that the AQP4 Ab serological status does not confer a specific clinical or paraclincal profile between seropositive and seronegative patients [Marignier *et al.* 2007]. Finally, there are no currently available data suggesting that seronegative patients should be treated differently from seropositive ones.

Conclusion

The identification of AQP4 Abs as a specific marker for DNMO spectrum and its pivotal role in DNMO pathogenesis has opened new areas in DNMO therapeutic research. In this review, we have tried to list the most promising among them. They still remain to be validated. The recent development of promising animal models of DNMO [Saadoun *et al.* 2010; Bennett *et al.* 2009; Bradl *et al.* 2009; Kinoshita *et al.* 2009] should be of great help.

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Conflict of interest statement

None declared.

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