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Review of Animal Models of Neuromyelitis Optica

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Abstract

Neuromyelitis optica (NMO) is a recurrent neuroinflammatory disease of the optic nerves and spinal cord associated with the anti-aquaporin-4 (AQP4) antibody biomarker, NMO-IgG. As clinical and scientific research interest in NMO grows, the need for an animal model becomes more urgent. Over the past few years, several groups have developed rodent models that partially represent human NMO disease. Passive transfer of the NMO-IgG is not pathogenic alone, but in certain contexts can recruit granulocytes and lead to increased inflammation. Studies of the cellular immune response against AQP4 have also shed light on the roles of B and T cells in NMO, especially focusing on the role of Th17 T helper cells. This review discusses the contribution of the available NMO animal models to the understanding of NMO disease pathogenesis.

Keywords

Neuromyelitis optica; Aquaporin-4; Experimental Autoimmune Encephalomyelitis; NMO-IgG

Introduction

Neuromyelitis optica (NMO) is a recurrent inflammatory disease that preferentially targets the optic nerves and spinal cord leading to blindness and paralysis (Eckstein, Saidha et al. 2011). NMO is unique among inflammatory diseases of the central nervous system (CNS) in that 33–91% of patients worldwide test positive for the NMO-IgG biomarker (Jarius and Wildemann 2010), an antibody that targets the aquaporin-4 (AQP4) water channel on the endfeet of astrocytes (Lennon, Kryzer et al. 2005). The discovery of the NMO-IgG in 2004

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(Lennon, Wingerchuk et al. 2004) created a surge of research publications on the prospect that the NMO-IgG may be pathogenic in inducing or exacerbating the disease by binding to AQP4 in susceptible CNS tissues (Roemer, Parisi et al. 2007; Saini, Fernandez et al. 2010). Building on decades of research on rodent models of multiple sclerosis, several groups have turned to rats and mice to understand the pathogenesis of NMO with specific questions about the roles of the NMO-IgG and AQP4. While no single rodent model has proven to be a perfect representation of NMO in humans, each study has contributed insights into the unique mechanism of NMO disease. The purpose of this review is to summarize these efforts.

Pathology of Human Disease

The goal of animal models of NMO is to reproduce all or part of the pathologic features of NMO disease in humans. In NMO autopsy examinations, lesions were initially described as demyelinating across a longitudinally extensive area of the spinal cord and associated with cavitation, necrosis and axonal pathology (Lucchinetti, Mandler et al. 2002). The necrotic features of these lesions may be an indicator of older lesions, since most demyelinated lesions show loss of myelin and astrocyte markers, but little necrosis (Parratt 2010, Matsuoka 2011). Features of innate and humoral autoimmunity are evidenced by infiltrating granulocytes including neutrophils and eosinophils, perivascular IgG and IgM deposition and presence of C9neo indicating complement fixation (Lucchinetti, Mandler et al. 2002). Interestingly, eosinophilic involvement is less prominent in Asian cases of optico-spinal MS (Ishizu et al 2005), leading some to question the importance of these cells in lesion development. Similarly, complement deposition occurs in restricted areas, as a thin rim around affected blood vessels corresponding to the “glial limiting membrane” (Parratt et al 2010), mirroring macrophage-laden perivascular cuffs (Parratt et al 2010, Lucchinetti et al 2002), and at pial and nerve root zones (Parratt 2010). Immunoglobulin deposition extends farther into demyelinated lesions than complement fixation, so the pathological impact of anti-Aqp4 antibody may involve complement-independent mechanisms as well. Macrophages and lymphocytes become prominent in lesions with myelin destruction, with histological evidence suggesting the former are clearing debris from degenerating astrocytes and oligodendrocytes.

Expression of AQP4, the target of the NMO-IgG, within lesions and in the normal CNS tissue of humans with NMO has been studied by several groups. The distribution of the AQP4 water channel in NMO lesions is distinct from what is seen in multiple sclerosis (MS) lesions. In MS, AQP4 increases or becomes diffusely distributed in and around actively demyelinating lesions but is generally absent in older inactive lesions (Roemer 2007, Parratt 2010). Numerous glial fibrillary acidic protein (GFAP)-positive large cell bodies tend to reside in demyelinating MS plaques. In contrast, AQP4 expression is absent in NMO lesions, regardless of stage of demyelination (Roemer, Parisi et al. 2007). The early lesions in the spinal cord, optic nerve and brain stem of NMO patients which have intact though often abnormal-appearing myelin express little to no AQP4, and account for approximately 10% of all lesions (Parratt 2010, Roemer 2007). GFAP is absent in some lesions and preserved in others, while a portion of the subacute demyelinated lesions appear to partially repopulate with GFAP+/AQP4− precursor-like cells (Parratt 2010). It should be noted that, even in a single patient, significant lesion heterogeneity can exist (Matsuoka et al 2011, Kira et al 2011): some lesions can resemble MS-like patterns of staining with intact AQP4 while others show complete loss of AQP4 and still others, loss of GFAP and AQP4. AQP4 loss has also noted in Balo’s disease despite the absence of an anti- AQP4 antibody (Matsuoka, Suzuki et al. 2010). This heterogeneity has led to conflicting ideas regarding to what extent anti-AQP4 antibodies, AQP4 loss or down-regulation contribute to overall disease. Since the area of AQP4 loss can exceed the area of myelin or GFAP loss, the evidence suggests that

AQP4 dysfunction caused by anti-AQP4 antibody leads to astrocyte death, followed by secondary destruction of myelin. The precise pathology of AQP4 in human NMO is still being investigated and animal models of NMO have focused on the highly specific NMO-IgG and its target, AQP4, as major players in the pathogenesis of disease.

Roles of NMO-IgG and Aquaporin-4 in Disease Pathogenesis

The roles of the NMO-IgG and AQP4 in NMO disease pathogenesis are still debated in the literature (Frohman and Kerr 2007), but there is circumstantial evidence that NMO-IgG is pathogenic in humans. The presence of the NMO-IgG in patients with transverse myelitis or optic neuritis confers a high likelihood of recurrence and development of NMO compared to similar patients who test negative for the NMO-IgG (Weinshenker, Wingerchuk et al. 2006). There is also some evidence that the NMO-IgG titer increases before an acute attack and declines with treatment and remission (Takahashi, Fujihara et al. 2007).

In models focusing on the pathogenicity of the NMO-IgG, targeting of AQP4 by an aberrant antibody presumes the destruction occurs after the antibody crosslinks across several AQP4 in susceptible CNS tissue. This, in turn, leads to complement fixation and consequent humoral-mediated inflammation (Saadoun, Waters et al. 2010). But in addition to humoral responses, recent studies have focused on the role of T-cells in NMO and how AQP4 processing by the immune system may lead to a targeted attack by the cellular immune system in patients with NMO, regardless of the presence or absence of NMO-IgG. Emerging NMO animal models focusing on the role of the AQP4 molecule in triggering a cellular response will be reviewed here

As yet, no single animal model has recapitulated all of the clinical and pathologic features of NMO disease in humans. This review will highlight the lessons learned from each model and propose future studies that may lend more insight into the NMO disease process.

Pathological Potential of NMO-IgG by Passive Transfer

In order to passively transfer pathogenic NMO-IgG to wholly create an NMO animal model, the human antibody must be able to react with the target antigen of the animal model species being considered, and the downstream immunologic activation must be reasonably similar between humans and the animal.

Three recent studies demonstrate that intraperitoneal injection of NMO-IgG into rats is not sufficient to create NMO-like lesions, even with access to the CNS. However, NMO-IgG appears to exacerbate inflammation and modulate the immune response in the context of experimental autoimmune encephalomyelitis (EAE).

To initiate EAE disease using active immunization, Lewis rats are injected with guinea pig myelin basic protein (gpMBP) emulsified with complete Freund’s adjuvant. By doing so, the blood-brain barrier (BBB) is compromised and MBP-reactive T cells enter the spinal cord (Gold, Linington et al. 2006). To address the issue of NMO-IgG pathogenicity in NMO, the rats received an intraperitoneal injection of the purified IgG from NMO-IgG seropositive patients at the peak of EAE disease. The anatomical location of EAE lesions is dictated by incoming T cells, with the disrupted BBB facilitating entry of circulating human antibodies primarily into the spinal cord. This model of EAE is particularly suited to these NMO studies, because although infiltration of T lymphocytes and macrophages leads to paralysis in EAE, there are typically no pathological changes of myelin or axon damage on pathology. Therefore, any residual neuropathological damage can be attributed to the infused patient IgG.

This combined EAE-passive transfer rat model resulted in complement activation, immunoglobulin deposition, and granulocyte influx in perivascular areas of the spinal cord (Kinoshita, Nakatsuji et al. 2009) similar to pathology of human NMO lesions (Lucchinetti, Mandler et al. 2002). In addition, AQP4 and astrocyte-specific glial fibrillary acidic protein (GFAP) immunoreactivity was severely depleted around inflamed blood vessels, also in line with what is observed in the human disease. The pattern of loss at the lesion edge suggests AQP4 is lost first, followed by GFAP; this sequence of events has also been proposed to occur early in the development of NMO lesions (Parratt and Prineas 2010). Swollen astrocytes are frequently noted in these lesions, suggesting the astrocyte is experiencing unregulated osmotic stress. This pathology was associated with an acute worsening of behavioral signs during the 96 hour post- NMO-IgG treatment interval (Kinoshita, Nakatsuji et al. 2009). While this model reproduces some key pathological features, one major limitation is the lack of injury to myelin. However, the lack of myelin damage in NMO animal models could also be explained by the acute nature of this passive transfer model. Passive transfer of a monoclonal antibody to MOG can produce widespread demyelinated lesions within the same timeframe used for these studies (Linington et al 1988), but demyelination secondary to astrocyte depletion may require additional time. The overall affinity of the transferred antibodies and their titer in the antibody preparation used for such studies may also be important. These studies employed IgG derived from serum that are polyclonal preparations, with only a small portion of the total IgG likely being toxic to astrocytes. The polyclonal NMO-IgG preparation are likely to be less potent than high affinity commercial anti-MOG monoclonal antibodies. In humans where AQP4 antibodies are produced continuously, astrocyte loss in lesions may result in chronic loss of trophic support to myelin. It remains to be seen if a short term loss of astrocytes in these animal models will injure myelin or if astrocytes will re-populate the lesion before such damage can occur.

Interestingly, other than to disrupt the BBB, T cells in EAE may not be necessary in the NMOIgG passive transfer model of NMO. Treatment with complete adjuvant (CFA) alone, which disrupts the blood brain barrier but causes very little T cell infiltration and no behavioral disease, was sufficient to promote lesion development with passive transfer of NMO-IgG (Kinoshita, Nakatsuji et al. 2010). This report found that one NMO-IgG sample which was exceptionally toxic to astrocytes by complement-mediated lysis *in vitro* caused lesions when passively transferred to CFA-treated Lewis rats, and all 3 samples tested resulted in perivascular astrocytic ballooning. This toxicity is relatively limited compared to that which occurs in the presence of T cells, but these results suggest that BBB disruption within a pro-inflammatory environment, and not T cells themselves, can affect astrocyte toxicity via pathogenic anti-AQP4 antibodies. Variable responses to individual antibody preparations suggest that, while many individual clones of antibodies in an individual patient may bind to astrocytes, not all necessarily kill these cells with the same potency or by the same mode of action (Bennett, Lam et al. 2009; Kinoshita, Nakatsuji et al. 2010). Conceivably, some patients likely have varying proportions of these more cytotoxic clones in their AQP4-reactive IgG repertoire than others.

An NMO animal model based on the adoptive transfer of EAE by encephalogenic T cell lines has also been developed (Bradl, Misu et al. 2009). The myelin-reactive T cells harvested from an immunized rat are stimulated repeatedly *in vitro*, creating a very narrow repertoire of MBP-reactive cells. Upon injection of these cells into a naïve rat, T cells and macrophages infiltrate the spinal cord and this results in paralysis (Bradl, Misu et al. 2009). Previous passive transfer studies in the context of adoptively transferred EAE have shown that addition of myelin-specific antibodies can exacerbate the disease and lead to myelin and axonal pathology (Linington, Bradl et al. 1988). By substituting the myelin-specific antibodies with AQP4-reactive NMO IgG, a different damaging pathology results. Again,

the anatomical location of lesions is driven by the preference of the T cells for the spinal cord and optic nerve, not the specificity of the antibody being used (Bradl, Misu et al. 2009). As seen with the active immunization model described above, AQP4 and GFAP immunoreactivity are depleted around complement-IgG+ blood vessels and granulocytes make up a significant proportion of the perivascular infiltrate. For reasons that are not clear, myelin damage is not apparent within 24–48 hours of the post-NMO-IgG treatment. Interestingly, IgG from MS or anti-AQP4-negative patients does not promote lesion development. The latter result suggests that either there is no other potential autoantibody in anti-AQP4-negative sera or that autoantibodies present do not sufficiently cross-react with rat proteins to be pathogenic.

As with active immunization, behavioral signs worsened in adoptive transfer EAE from an EAE paralysis score of 2.0 to a 3.0 (i.e. progressing from weakness in the hind limbs to complete paralysis of the hind limbs) just 24 hours after NMO-IgG injection (Bradl, Misu et al. 2009). To attempt to mirror the T cell responses that might be occurring in humans with NMO, a variation of the adoptive transfer model was designed with AQP4-specific T cells in place of MBP-reactive cells to facilitate entry of circulating NMO-IgG in Lewis rats (Pohl, Fischer et al. 2011). Active immunization with T cell epitope-containing domains of AQP4 did not result in appreciable inflammation in the CNS. However, with repeated stimulation with AQP4 antigen in culture, cell lines to these AQP4 peptides did acquire some ability to enter the CNS but still resulted in no astrocyte or myelin damage when adoptively transferred alone. When AQP4-reactive T-cells were transferred along with NMO-IgG, inflammatory lesions were larger and AQP4 loss was readily observed (Pohl, Fischer et al. 2011). In rodents, these T cell lines also led to subclinical inflammation of other AQP4-expressing organs, including the kidney and muscle, and this was exacerbated by co-injection of NMO-IgG.

This study highlights an important unresolved issue in NMO pathogenesis. Only the CNS is damaged by NMO disease, but NMO-IgG has access to peripheral AQP4-bearing tissues (Lennon, Kryzer et al. 2005; Pohl, Fischer et al. 2011). It has been proposed that a specific conformation or isoform may be enriched in the optic nerve and spinal cord to account for the distribution of lesions in NMO, while other brain regions or peripheral organs are spared (Saini, Fernandez et al. 2010). However, adsorption of NMO-IgG to AQP4-expressing cells before infusion can dampen its pathogenic potency (Bradl, Misu et al. 2009), arguing against this mechanism. Alternatively, binding of the NMO-IgG to AQP4 may result in endocytosis of the antibody-antigen complex or lead to complement fixation, which may, in turn, be a more damaging event in the CNS than in other organs. Other organs might express more efficient complement regulatory mechanisms or other aquaporins which compensate for the dysfunction or loss of AQP4. Differential recruitment of inflammatory cells to particular CNS or peripheral locations may also explain the distribution of lesions. For example, EAE mediated by Th1 T-helper cells recruits fewer granulocytes than EAE polarized to Th-17 T helper cells and use different adhesion molecules to enter into the CNS (Rothhammer, Heink et al. 2011). In addition, the makeup of the infiltrating cells also depends on expression of particular adhesion molecule usage as CCR2-deficient mice, which cannot recruit peripheral monocytes into the CNS during EAE, recruit neutrophils in their place (Saederup, Cardona et al. 2010).

A third model of passive transfer of NMO-IgG involved injection of NMO-IgG directly into the mouse brain (Saadoun, Waters et al. 2010; Saadoun, Waters et al. 2011). This approach is useful for examining the direct effect of NMO-IgG binding within the CNS of an intact animal without immunization. Lesion development required the co-injection of human (but not mouse) complement and resulted in AQP4 loss, astrocytic swelling, perivascular complement deposition and granulocyte infiltration. In contrast to rat models, myelin was

also damaged. This study further demonstrated that human NMO-IgG could not activate mouse complement *in vitro*. By the same token, inhibitors of mouse complement activation, which are expressed in the central nervous system, may not be active towards human complement. Thus, this cross-species incompatibility may promote lesion development by human complement and IgG in the mouse brain.

Pathologic Potential of Anti-AQP4 Antibodies by Immunization

The NMO-IgG has been demonstrated in both *in vivo* and *in vitro* models to be pathogenic, as described above. However, the question of how such toxic anti-AQP4 antibodies arise has not been addressed. Circulating anti-AQP4 antibodies can be generated *de novo* by immunizing rodents with whole AQP4 or peptides (Kalluri, Rothhammer et al. 2011). An NMO animal model using AQP4 protein to raise pathogenic anti-AQP4 antibodies has not been published. We have found that Lewis rats can produce high titers of antibodies against extracellular epitopes of human AQP4. However, even in the context of EAE, these antibodies do not modulate the EAE immunopathogenic response (unpublished observations, ML). Similarly, C57BL6 mice can generate antibodies against full length AQP4 that bind AQP4 in cell based assays, but these animals do not develop illness (Kalluri, Rothhammer et al. 2011). Generation of cytopathic, conformationally-dependent antibodies in animal models remains a challenging hurdle to understanding the source of the NMO-IgG in humans.

Cellular Immune Responses Against AQP4

Two strong arguments for the involvement of T-cells in the NMO disease process include the necessity of T-cells for IgG class switching and the requirement for T-cells in some of the passive transfer NMO animal models described above. From these animal experiments, it appears that T-cells do not have to be specific for AQP4 to facilitate passive transfer to NMOIgG in rodents.

Antigen-specific T cell responses driving NMO in humans remain to be characterized.

T cells promote immunoglobulin class switching but may perform other “helper functions” that exacerbate NMO. Knowledge of these cells’ fine antigenic specificity will help generate T cell help for animal model development. More importantly, identification of immunodominant epitopes of AQP4 in animal models could guide similar studies in humans which could use these epitopes to anergize the T cells to treat NMO, as has been proposed for MS (Kohm et al 2005).

Two groups recently investigated the precise AQP4 epitope that can stimulate T-cells. Following immunization with full-length human AQP4, Nelson et al (2010) examined mouse T cell responses to both human and mouse AQP4 peptides and Kalluri et al (2011) used slightly different overlapping peptide fragments to investigate T cell responses. Immunization with protein or peptides did not result in any behavioral disease. However, these studies identified several T cell-responsive peptides (Table 2) of which $AQP4_{21–40}$ was determined to be the immunodominant epitope that triggered production of interleukin-17 (Nelson, Khodadoust et al. 2010). Kalluri et al (2011) found that T cell lines derived from $AQP4_{22–36}$ –immunized mice produced interferon-gamma (IFNγ), interleukin-4 and interleukin-10 (Kalluri, Rothhammer et al. 2011). The Kalluri study went on to show that immunization with either the immunodominant peptide or the full-length protein did not alone induce histological disease. Nevertheless, these immunological studies are the first step for building an NMO model that includes T-cell mediated activity directed against AQP4.

The involvement of T cells in NMO may extend beyond peripheral helper functions. In particular, the importance of Th17 T helper cells in NMO is supported by two key observations. First, the frequency of the Th17 subtype is elevated in NMO relative to MS (Wang, Dai et al. 2011), as are CSF levels of IL17 (Ishizu, Osoegawa et al. 2005) and the downstream neutrophil-recruiting cytokine, IL8 (Uzawa, Mori et al. 2010). Second, in contrast to MS, NMO and Th17-driven EAE are exacerbated by interferon-beta (IFNβ) (Axtell, de Jong et al. 2010). EAE studies suggest this subset of cells may direct the tendency of lesions to localize to the optic nerve and spinal cord of NMO patients, a role that goes beyond peripheral helper functions. Depending on the ratio of Th1 to Th17 cells in EAE and the adhesion molecules used by these cells, inflammatory cells can be directed to infiltrate the optic nerve more severely than would Th1 cells alone (Stromnes, Cerretti et al. 2008; Saederup, Cardona et al. 2010; Rothhammer, Heink et al. 2011; Herges, de Jong et al. 2012). Compared to the Th1-driven disease used extensively to model MS, Th17-driven animal models involve heavy recruitment of granulocytes into the CNS. Intriguingly, this inflammatory pattern and associated behavioral signs can be attenuated by treatment of these EAE mice with the neutrophil elastase inhibitor, Sivelestat (Herges, de Jong et al. 2012). Animal models based on cellular immune responses, which are not driven by AQP4 antibodies, may provide insight in to the pathogenesis of seronegative NMO disease.

Other NMO animal models

Other NMO-like animal models have been published that do not involve NMO-IgG or AQP4. Still, they resemble NMO in either their localization of disease to the optic nerves and spinal cord or they resemble in NMO in their tissue pathology.

While most models of EAE generally target the spinal cord, the genetic “2D2” EAE model was developed in which mice born with T cells directed against myelin oligodendrocyte glycoprotein (MOG35–55) were crossed with mice that have a transgenic B cell receptor to MOG (Bettelli, Baeten et al. 2006; Krishnamoorthy, Lassmann et al. 2006). These mouse progeny spontaneously develop optic neuritis and severe inflammatory spinal cord lesions with relative sparing of the brain as in NMO disease in humans. However, unlike human NMO pathology, there was no evidence of complement deposition or granulocyte recruitment in this model. Interestingly, mice housed under conventional conditions (non “pathogen-free”) develop lesions which include granulocytes, especially eosinophils (Krishnamoorthy, Lassmann et al. 2006). The infiltrating T helper cells were mainly of the Th1 type (Bettelli, Baeten et al. 2006; Krishnamoorthy, Lassmann et al. 2006), but whether the role of Th17 cells might be more prominent for mice raised under conventional conditions that promote granulocyte recruitment was not examined in this study (Krishnamoorthy, Lassmann et al. 2006).

Considerations for Animal Models for Anti-AQP4-Seronegative NMO

The argument against an AQP4-specific pathogenic IgG as the cause of NMO is the description of an identical clinical and pathological disease in NMO patients who test negative for the antibody. In addition, detectable AQP4 seropositivity sometimes does not develop until years after NMO progression (Kira 2011).

An animal model for damage that occurs in AQP4-seronegative patients is desirable. Several theories have been put forth to explain how individuals without measureable anti-AQP4 titers can have a disease phenotypically indistinguishable from anti-AQP4 seropositive cases. A potential explanation is that, like with myasthenia gravis, a subset of patients produce autoantibodies targeting proteins other than AQP4 (Hoch, McConville et al. 2001). One study found that 7–20% of individuals with seronegative NMO produced antibodies to MOG (Mader et al 2011). Autoantibodies to proteins closely associated with AQP4 on

astrocyte membranes, such as the inward-rectifying potassium channel 4.1 (Kir4.1) is a possibility. It should be noted that plasmapheresis is effective even for seronegative NMO and lesions in seronegative NMO samples show evidence of a humoral-mediated disease similar to seropositive samples. However, IgG from these individuals appears to have limited toxicity towards astrocytes *in vitro* (Sabater et al 2009) or myelin in vivo (Bradl et al 2009).

Another explanation for seronegative NMO is that NMO is primarily a T cell mediated disease, in which Th17-producing cells are the master inflammatory regulators (Ishitzu 2005). That could also explain why drugs targeting Th1 diseases (Natalizamab, IFNβ) are ineffective or exacerbate NMO (Axtell 2010). In this model, autoantibodies developed in the wake of tissue destruction may then exacerbate disease even if they do not initiate irreparable damage alone (Kira 2011).

Like MS, NMO may be mediated by a variety of independent and overlapping disease mechanisms. Anti-AQP4-positive and seronegative disease may be mediated by different immunological pathways (Icoz et al 2010). Animal models based on both antibody-dependent and antibody-independent mechanisms may answer some of these questions.

Future Directions

Using knowledge of EAE as a guide, animal models of NMO are developing quickly. No one model is expected to hold all the answers to the pathogenesis of NMO, but by combining passive transfer and active immunization models, the individual pathological events in NMO lesions and perhaps the order in which they occur can be further understood. With this knowledge in hand, therapeutic strategies to minimize and repair tissue damage in the CNS of NMO patients can be designed.

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Mult Scler Relat Disord. Author manuscript; available in PMC 2014 February 17.

Table 1

Reference	Animal	Model system	Aqp4 loss?	GFAP loss	PMNs	Myelin Loss?	Behavioral	Time point examined post transfer	notes
Bradl et al 2009	Lewis rat	MBP T cell line co-transfer with NMO IgG	yes	yes	Yes	No	Yes; 24hrs p.t., scores went from 2.0 in ctrls to 3.0	24hrs	No lesions without T cells; leaky BBB (juvenile animal, adult CVOs) are spared
Pohl et al 2011	Lewis rat	AQP4-specific T cell line to $AQP4_{207–232}$ + NMO-IgG	yes	ND	ND	No	ND	24hrs post-transfer of IgG	Meningeal lesions; non-pathogenic kidney inflammation
Kinoshita et al 2009	Lewis rat	MBP immunization + NMO IgG ip each of 4 days after peak disease	yes	yes	Yes	ND	Yes; score higher than ctrls for the 4 days	5 days after first NMO-IgG inj	Edges of lesion have intact GFAP, but swollen astrocytes
Bennett et al 2009	Lewis rat	Rec. human Ab into MBP EAE rats	ND	yes	ND	No (though some abnormalities)	ND	30 hours	Only hu. rec. Abs that x-react with rat AQP4 caused this pathology
Kinoshita et al 2010	Lewis rat	CFA only, ip inject Ig 3d later for consecutive 4 days	Yes, in 1 of 3 NMO-IgG sampl	Yes, in 1 of 3	Yes	ND	No behavioral signs with only CFA	5 days after NMO IgG injection	All 3 samples killed rat ast in vitro with hu complement, all 3 caused swollen astrocytes in vivo; only 1 sample caused astrocyte depletion
Saadoun 2010	CD1 mice	IgG+ complement Intraventricular or intracerebral (striatum) Several injections	yes	yes	“few”	yes	Y-tunnel assay; lesioned animals prefer ipsilateral decision	AQP4 loss as early as 12hrs; inflammation builds later	Mouse complement was not fixed by NMO-IgG in vivo or in vitro
Saadoun 2011	Mice vs nude mice	IgG+complment external capsule several injections	yes	ND see Saadoun 2010)	Yes	yes	ND (see Saadoun 2010)	24hrs after 1st inj or 48hrs after 2nd	Nude mice lack mature T cells; have sl more PMNs than wt mice; AQP4KO intact

Table 2

Ref	Animal	Immunized with	Immunogenic	Comments
Pohl et al 2011	Lewis rats	207–232,277–322, or 296–304	207–232 296–304 (milder)	Cell lines, not peptide immunization directly, caused subclinical inflammation in the CNS w/o AQP4 loss
Nelson et al 2010	C57Bl6, SJL	Full length human AQP4, peptides	21–40; several others	Peptide screen; Both strains; T cell response with either full length or peptide
Kalluri et al 2011	C57Bl6	Full length human AQP4	22–36 82–108 139–153 211–303	Peptide screen