

Opticospinal multiple sclerosis in Japanese

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Abstract

Antibodies to aquaporin-4 (AQP4) are found in a number of Japanese opticospinal multiple sclerosis (OSMS) patients. Whether anti-AQP4 antibody-positive and -negative OSMS patients are afflicted with an identical disease remains unknown. To clarify immunological differences between the two groups of patients, we studied serum antibody titres against AQP4 in 191 patients with idiopathic central nervous system demyelinating diseases and clarified any relationships with immunological parameters. The anti-AQP4 antibody positivity rate was higher in patients with OSMS (36.2%), idiopathic recurrent myelitis (23.5%), and recurrent optic neuritis (26.9%) than in conventional MS patients (8.0%), and those with other diseases (0%). Anti-AQP4 antibody titre was significantly higher in patients with SS-A/B antibodies than in those without. Anti-AQP4 antibody-negative OSMS patients showed significantly higher CD4⁺IFN- γ ⁺IL-4⁺T cell percentages and intracellular IFN- γ /IL-4 ratios than anti-AQP4 antibody-positive patients, anti-AQP4 antibody-negative conventional MS patients, and healthy controls. As well, CD4⁺IFN- γ ⁺IL-4⁺T cell percentages were negatively correlated with anti-AQP4 antibody titres. In CSF, OSMS patients had significantly higher levels of IFN- γ and granulocyte colony-stimulating factor levels than patients with non-inflammatory neurological diseases and other causes of myelitis. A significant increase of IL-17 compared with non-inflammatory neurological diseases patients was only found in OSMS patients, irrespective of the presence or absence of anti-AQP4 antibody. These findings suggest that high titres of anti-AQP4 antibodies are produced as a result of heightened humoral autoimmunity, and that they are likely to contribute to extensive lesion development through disturbed resolution of vasogenic oedema. Moreover, since intrathecal up-regulation of IL-17 and IFN- γ is characteristic of OSMS, Th17/Th1 cells may be critical for the initiation of inflammation and the disruption of blood-brain barrier (BBB); rendering anti-AQP4 antibody get across the BBB.

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) whereas neuromyelitis optica (NMO) is an inflammatory disease of the CNS selectively affecting the optic nerves and spinal cord. The pathological hallmark in MS is sharply demarcated demyelinating plaques with axons relatively preserved. In NMO, both axons and myelin are destroyed resulting in necrotic cavitation. Although MS is rare in Asians; when it does appear, selective but severe involvement of the optic nerves and spinal cord is characteristic.¹ This form, termed opticospinal MS (OSMS), has similar features to the relapsing form of NMO in Western populations. Although the nosological position of NMO has long been a matter of debate, the recent discovery of a specific IgG against NMO, designated NMO-IgG², suggests that NMO is a distinct disease entity with a fundamentally different aetiology from MS. Because NMO-IgG has been reported to be

present in about 50 to 60% of OSMS patients with longitudinally extensive spinal cord lesions (LESCLs)^{2,3}, OSMS in Asians has been suggested to be the same entity as NMO.⁴ However, it remains to be elucidated whether patients with or without these antibodies are inflicted with an identical disease.

NMO-IgG/ANTI-AQP4 ANTIBODY POSITIVITY RATE

There are discrepancies in the detection rates for anti-AQP4 antibody among the Japanese patient series of Takahashi et al⁵ (20/22, 90% in NMO patients), Tanaka et al⁶ (60% in OSMS patients with LESCLs) and Matsuoka et al⁷ (11/31, 35% in OSMS patients with LESCLs). The reasons might relate to differences in subjects (NMO or OSMS with LESCLs; selected or consecutive patients; northern or southern Japanese patients, who have been reported to have somewhat different clinical and laboratory features according to a

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recent nationwide survey)⁸, and in the methods (AQP4-transfected or GFP-AQP4 fusion protein-transfected; fixed transfected cell specimens or unfixed ones; 1:4 dilution or 1:400 dilution). We recently increased sensitivity (100% sensitive against NMO-IgG) by using unfixed preparations of GFP-AQP4 fusion protein-transfected cells and 1:4 diluted sera; however, the results were practically the same. Using this method, we found that the anti-AQP4 antibody positivity rate was higher in patients with OSMS (19/58, 32.7%), idiopathic recurrent myelitis (4/17, 23.5%), or recurrent optic neuritis (7/26, 26.9%) than in conventional MS (CMS) patients (6/75, 8.0%) and others (0/87). A similar positivity rate was reported in NMO patients (16/48, 33.3%) in Cuba and the French West Indies.⁹

CLINICAL AND NEUROIMAGING FEATURES

As compared with anti-AQP4 antibody-negative CMS patients, anti-AQP4 antibody-positive MS patients showed significantly higher frequencies of severe optic neuritis, acute transverse myelitis, and LESCLs while most conditions were also common to anti-AQP4 antibody-negative OSMS patients.⁷ The LESCLs in anti-AQP4 antibody-positive patients were preferentially located at the upper-to-middle thoracic cord, while those in anti-AQP4 antibody-negative OSMS patients appeared throughout the cervical-to-thoracic cord. On axial planes, the former predominantly showed central grey matter involvement while holocord involvement was predominant in the latter. By contrast, LESCLs in anti-AQP4 antibody-negative CMS patients mainly involved the mid-cervical cord, presenting a peripheral white matter-predominant pattern as seen in short spinal cord lesions. Anti-AQP4 antibody-positive MS patients fulfilling definite NMO criteria showed female preponderance, higher relapse rate, greater frequency of brain lesions, and less frequent responses to interferon beta-1b than anti-AQP4 antibody-negative OSMS patients with LESCLs. Multiple logistic analyses revealed that the emergence of the anti-AQP4 antibody was positively associated with only a higher relapse rate, not with optic-spinal presentation or LESCLs. These findings suggested that LESCLs are distinct according to anti-AQP4 antibody positivity and clinical phenotypes. There are cases of anti-AQP4 antibody-positive MS/NMO that are distinct from CMS and anti-AQP4 antibody-negative OSMS with LESCLs in Japanese. This

indicates that the mechanisms producing LESCLs are heterogeneous even in patients with optic-spinal presentation, namely AQP4 autoimmunity-related and -unrelated.

We titrated the anti-AQP4 antibody and found the following. (1) Anti-AQP4 antibody titre was significantly higher in patients with SS-A/B antibodies than in those without, but was not significantly correlated with either EDSS score or disease phase. (2) Patients with a high anti-AQP4 antibody titre (≥ 512) showed significantly higher frequencies of extensive brain lesions and other autoantibodies/autoimmune diseases than anti-AQP4 antibody-negative OSMS patients with LESCLs. However, the former group paradoxically showed significantly lower frequencies of acute transverse myelitis and severe motor disability than the latter, despite the optic nerve impairment being equally severe between the two. (3) Extensive brain lesions in patients with high antibody titres showed a vasogenic oedema pattern on diffusion-weighted MRI. (4) Anti-AQP4 antibody-negative OSMS patients showed significantly higher CD4⁺IFN- γ ⁺IL-4⁺T cell percentages and intracellular IFN- γ /IL-4 ratios than anti-AQP4 antibody-positive OSMS patients, anti-AQP4 antibody-negative CMS patients and healthy controls, and CD4⁺IFN- γ ⁺IL-4⁺T cell percentages showed a significant negative correlation with anti-AQP4 antibody titres. (5) The phenotypic frequency of the *HLA-DPB1*0501* allele was significantly increased in anti-AQP4 antibody-positive patients compared with controls but not in anti-AQP4 antibody-negative ones. These findings suggest that high titres of anti-AQP4 antibodies are produced as a result of heightened humoral autoimmunity and the presence of the *HLA-DPB1*0501* allele in Japanese. As well, the high titres are likely to contribute to extensive lesion development through disturbed resolution of vasogenic oedema.

INTRATHECAL IMMUNE RESPONSES

To clarify differences in intrathecal immune responses among various inflammatory myelopathies, we used a fluorescent bead-based immunoassay to simultaneously measure 27 cytokines, chemokines and growth factors in CSF from 22 patients with atopic myelitis, 20 with OSMS, 11 with HTLV-1-associated myelopathy (HAM), 9 with Sjögren syndrome-related myelitis and 20 with other non-inflammatory neurological diseases (OND).¹⁰ In OSMS patients, IFN- γ and

granulocyte colony-stimulating factor levels were significantly higher than patients with OND and other causes of myelitis whereas in AM patients, eotaxin and IL-9 were significantly increased as compared with OND and other myelitis patients. A significant increase in IL-17 compared with OND patients was found only in OSMS patients, irrespective of the presence or absence of anti-AQP4 antibody. In HAM patients, IP-10 and RANTES were higher than in OND and other myelitis patients. In Sjögren syndrome-related myelitis patients, MIP-1 α and MIP-1 β were higher than in OND patients. These findings suggested that intrathecal up-regulation of IL-17/IFN- γ is characteristic of OSMS, which is distinct from the eotaxin and Th2 cytokines-related allergic inflammatory condition of atopic myelitis.

PROPOSED MECHANISM OF NMO

Lucchinetti *et al*¹¹ reported perivascular immune complex deposition (IgM, IgG and C9neo) in a rim or rosette pattern in CNS lesions from autopsied NMO patients. Mitsu *et al*¹² described an extensive loss of AQP4 accompanied by decreased GFAP-staining in active perivascular lesions where MBP-staining was relatively preserved in postmortem Japanese NMO cases. Roemer *et al*¹³ also made similar observations regarding novel NMO lesions in the spinal cord and medullary tegmentum that extended to the area postrema where the blood-brain barrier is absent; suggesting a primary role for anti-AQP4 antibody in NMO pathology. Based on the presence of immunoglobulin and complement deposition in active perivascular lesions, astrocytic impairment associated with the loss of AQP4 caused by humoral autoimmune attack is assumed to be the primary event in NMO. Anti-AQP4 IgG1 antibodies across the BBB bind to AQP4 molecules on astrocyte foot processes and activate complements. Activated complements mobilize neutrophils and eosinophils that then facilitate tissue destruction.

However, there are several concerns about this hypothesis. First, the deposited immunoglobulins in postmortem NMO lesions are mainly IgM, while the anti-AQP4 antibodies described are all IgG, not IgM. Second, in the presence of high titres of anti-AQP4 antibodies, some patients remain in remission.¹⁴ Since AQP4 is present in the astrocyte foot processes behind the BBB, additional factors that disrupt the BBB and allow the antibody to enter through the BBB may be necessary to initiate inflammation in the parenchyma. Third, AQP4 is present in distal collecting tubules and in gastric mucosa, and NMO-IgG binds to these

structures.¹⁵ However, no impairments in either kidney or stomach have been seen in such cases, suggesting that the presence of complement-fixing anti-AQP4 antibody is not enough to produce tissue damage. Fourth, AQP4 expression ubiquitously exists throughout the CNS, though its expression varies; being high in the grey matter of the spinal cord.¹² Such a ubiquitous presence cannot explain the selectiveness of lesion distribution; in the optic nerves and the spinal cord. Fifth, we have analysed serum CH50, C3, C4, and C-reactive protein (CRP) levels and their relationship to clinical phases in 118 MS patients with or without anti-AQP4 antibody.¹⁶ Serum CH50 levels were significantly higher in patients with anti-AQP4 antibody than in OSMS and CMS patients without anti-AQP4 antibody at relapse, but not in remission. The frequency of hypercomplementemia at relapse was also higher in patients with anti-AQP4 antibody than in anti-AQP4 antibody-negative CMS patients (70.4% vs. 29.0%, $P_{corr} < 0.05$). C3 and C4 levels did not differ significantly among the three groups at relapse. In patients with anti-AQP4 antibody, coexistence of both hypercomplementemia and high CRP values was more common at relapse than in the remission phase (36.0% vs. 10.5%, $P < 0.05$). Therefore, hypercomplementemia in anti-AQP4 antibody-positive patients seems to reflect a systemic inflammatory reaction at relapse; also, that complement consumption as seen in systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and Sjögren syndrome does not occur in anti-AQP4 antibody-positive patients.

OUR HYPOTHEISIS

In our series, more than half the anti-AQP4 antibody-positive patients had brain lesions that fulfilled the Barkhof criteria, while anti-AQP4 antibody-negative OSMS patients with LESCLs showed substantially fewer brain lesions.⁷ Moreover, some Japanese anti-AQP4 antibody-positive patients predominantly had brain lesions or even only brain lesions. Therefore, the anti-AQP4 antibody-positive condition does not completely overlap NMO or OSMS. We previously reported that IL-17 is up-regulated in the CSF of OSMS patients and that levels of both IL-17 and downstream cytokine IL-8 in CSF show a significant positive correlation with spinal cord lesion length.¹⁷ IL-17 is produced by Th17 cells, which belong to a distinct lineage from Th1 and Th2 cells.¹⁸ Th17 cells have come under much

attention because increasing evidence suggests that they, but not Th1 cells, are responsible for organ-specific autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE).^{18,19} IL-8 is a chemokine for neutrophils. In OSMS patients, CSF neutrophilia and infiltration of neutrophils to severely destructed lesions have been observed.¹⁷ Elevated IL-8 is assumed to be partly responsible for such neutrophil activation and mobilization in OSMS. Indeed, myeloperoxidase, an activated neutrophil product, is up-regulated in sera from OSMS patients, especially in those with LESCLs at relapse.²⁰ Th17 cells carrying granzyme B have recently been shown to efficiently disrupt BBB tight junctions and loosen the BBB.²¹ Therefore, autoimmune Th17 cells may initiate BBB disruption and inflammation in OSMS, causing vasogenic oedema in the CNS (Fig. 1).

Regarding other factors that possibly have effects on vascular permeability; we have reported that levels of vascular endothelial growth factor (VEGF) in sera are significantly increased in OSMS patients, showing a significant positive correlation with spinal cord lesion length.²² IL-17 has also been shown to induce VEGF production in target tissues.²³ Moreover, a mutation in the *platelet activating factor acetylhydrolase (PAF-AH)* gene, which inactivates its enzymatic activity of metabolizing PAF into an inactive form, is significantly more frequent in OSMS patients than healthy controls.²⁴ Indeed, PAF-AH activity in peripheral blood is decreased in OSMS patients²⁴, which likely prolongs PAF activity and increasing vascular permeability as well as vascular growth. These vascular-acting factors also enhance tissue oedema in OSMS (Fig. 1).

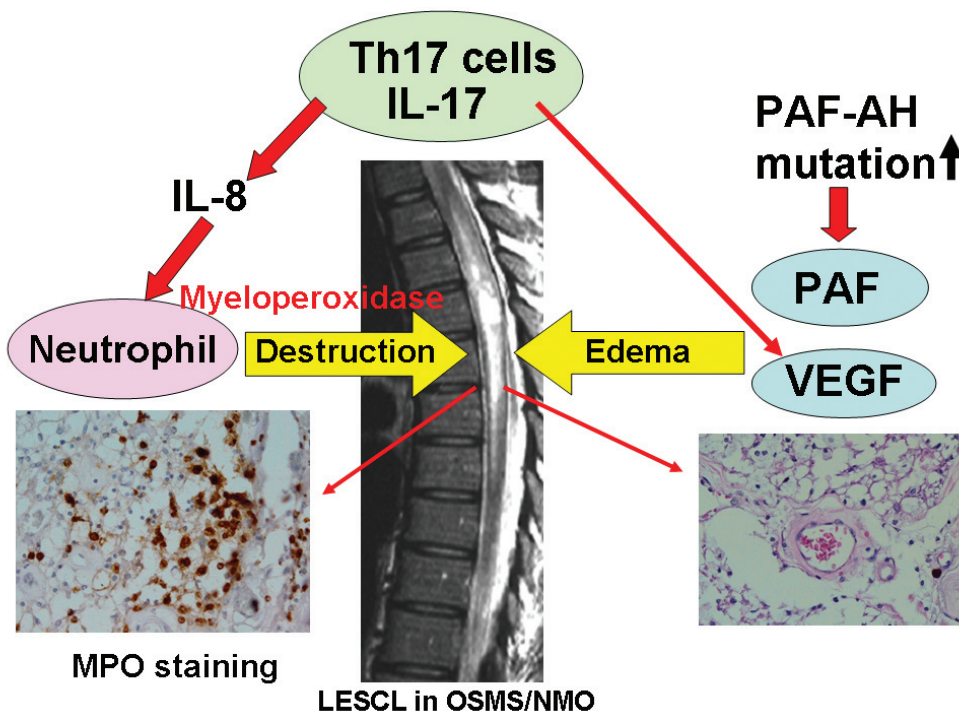


Figure 1. Th17 cell-mediated mechanism producing extensive spinal cord lesions in opticospinal multiple sclerosis (OSMS).

Autoimmune Th17 cells produce IL-17, which then induces IL-8 production in various cells in tissues. IL-8 activates and mobilizes neutrophils. In OSMS patients, serum myeloperoxidase levels are elevated, especially at relapse. Thus the IL-17/IL-8 axis may contribute to severe tissue destruction in OSMS. Platelet activating factor (PAF) acetylhydrolase (PAF-AH) metabolizes PAF to an inactive form. Mutation in the *PAF-AH* gene, which inactivates its enzymatic activity, is significantly more frequent in OSMS patients than healthy controls. Moreover, vascular endothelial growth factor (VEGF) is increased in sera from OSMS patients. It is possible that elevated VEGF and prolonged PAF activity increase vascular permeability and cause tissue oedema. MPO=myeloperoxidase.

As AQP4 knock-out mice showed prolongation of vasogenic oedema²⁵, but a decrease of cytotoxic oedema²⁶, anti-AQP4 antibody produced by heightened humoral autoimmune background or secondarily by tissue breakdown may prolong resolution of tissue oedema, thereby contributing to further tissue destruction in NMO/OSMS patients. NMO-IgG/anti-AQP4 antibody-positive patients show a significantly higher frequency of severe optic nerve damage as compared with anti-AQP4 antibody-negative CMS ones.^{3,7} We recently investigated multimodality evoked potentials (visual evoked potentials, motor evoked potentials and somatosensory evoked potentials) in 111 relapsing-remitting or relapsing-progressive MS patients, 18 of whom were seropositive for anti-AQP4 antibody. We found that more patients with anti-AQP4 antibody showed a lack of the P100 VEP component than those without the antibody (11/17, 64.7% vs. 20/84, 23.8%, $p=0.003$), whereas the frequency of delayed P100 latency was significantly higher in the latter group than in the former (1/17, 5.9% vs. 28/84, 33.3%, $p=0.021$). Optic nerves are especially vulnerable to the detrimental effects of tissue oedema in the optic

canal where space is tight and increased tissue pressure easily causes circulatory insufficiency. In the spinal cord, the thoracic cord is prone to develop LESCLs in anti-AQP4 antibody-positive patients as it acts as the watershed for vascular supply in the spinal cord. However, AQP4 is widely present from the cervical to sacral cord. Therefore, even in the spinal cord, vulnerability to ischaemia may be one factor contributing to the development of LESCLs. Prolongation of vasogenic oedema at sites where the surrounding space is tight or vascular supply is poor may cause poor recovery from tissue damage in patients with anti-AQP4 antibody.

Anti-AQP4 antibody may directly cause NMO or a disease distinct from either MS or NMO (anti-AQP4 autoimmune syndrome of the CNS). Alternatively, anti-AQP4 antibody is a secondary product that is produced following tissue destruction. Once produced it may modify the disease course as a functional autoantibody like anti-neurofascin antibody in chronic progressive MS (Fig. 2)²⁷, or could simply be an epiphenomenon without any function *in vivo*.

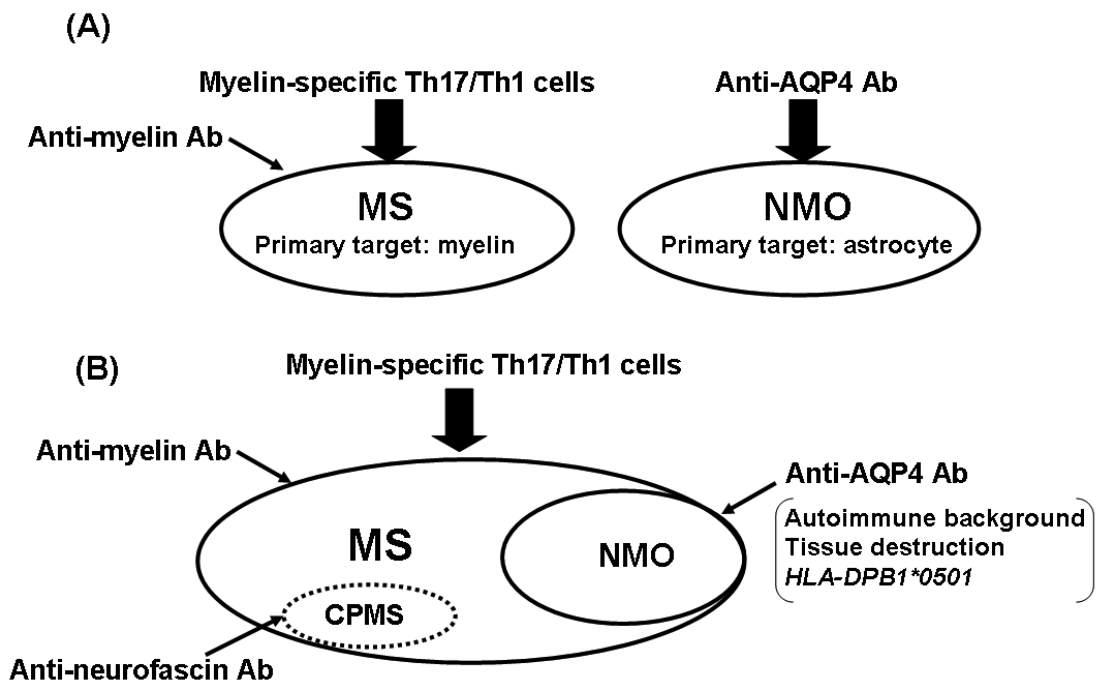


Figure 2. Two hypothetical mechanisms in MS and NMO. (A) Anti-AQP4 antibody directly causes NMO, which is distinct from MS. (B) Myelin-specific Th17/Th1 cells initiate CNS inflammation. Anti-myelin antibody produces myelin loss, anti-neurofascin antibody causes axonal loss and anti-AQP4 antibody induces astrocyte foot process destruction.

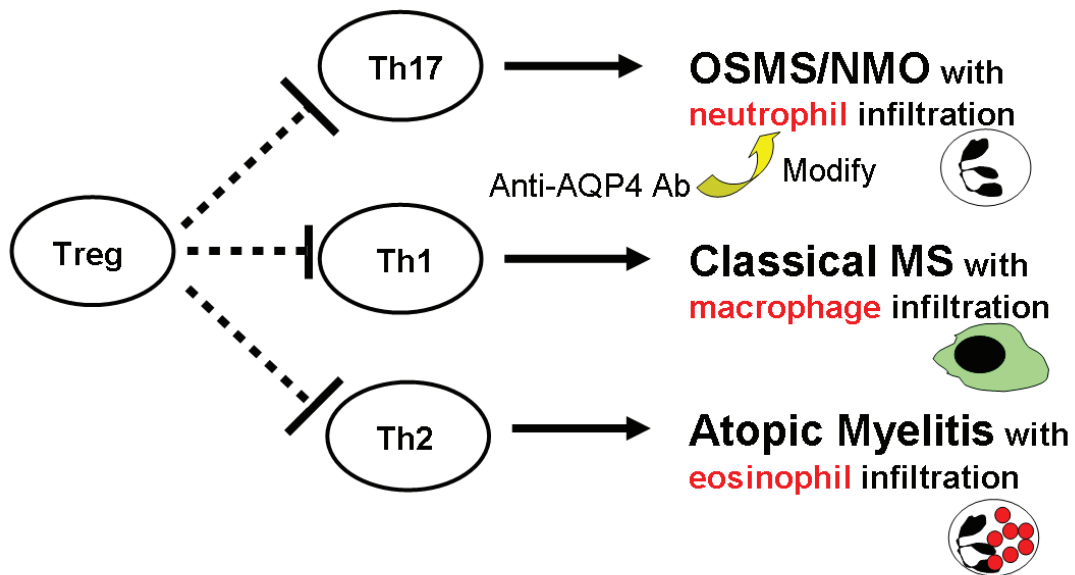


Figure 3. Distinct Th subsets produce distinct inflammatory (demyelinating) CNS disorders, when regulatory T cells (Treg) can not effectively suppress Th effector activities.

FUTURE PERSPECTIVES

It remains to be elucidated whether anti-AQP4 antibody-negative NMO/OSMS patients have the same disease as anti-AQP4 antibody-positive NMO/OSMS patients. If these patients do have the same disease, which is distinct from MS, then what is the actual cause or specific autoantigen? If AQP4 is one of the autoantigens, are there also other autoantigens responsible for seronegative NMO/OSMS? How is the anti-AQP4 antibody produced and how does the antibody get across the BBB to reach the astrocyte foot process? If the big picture of immunology produces a spectrum of idiopathic inflammatory (demyelinating) diseases of the CNS, Th17 cells may produce OSMS/NMO with or without anti-AQP4 antibody, Th1 cells may cause classical MS and Th2 cells may induce atopic myelitis (Fig. 4). There are many questions remaining to be answered.

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