

Loss of Aquaporin-4 in Active Perivascular Lesions in Neuromyelitis Optica: A Case Report

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MISU, T., FUJIHARA, K., NAKAMURA, M., MURAKAMI, K., ENDO, M., KONNO, H. and ITOYAMA, Y. *Loss of Aquaporin-4 in Active Perivascular Lesions in Neuromyelitis Optica: A Case Report.* Tohoku J. Exp. Med., 2006, **209** (3), 269-275 — Neuromyelitis optica (NMO) is clinically characterized by severe optic neuritis and transverse myelitis. In Japan, NMO has been named optic-spinal multiple sclerosis (OSMS) and it has been thought to be a subtype of multiple sclerosis (MS). However, several clinical and laboratory findings suggest NMO or OSMS is distinct from MS. Recently, the disease-specific antibody (NMO-IgG) was found in the serum from NMO patients, and its target antigen was identified as aquaporin-4 (AQP4) water channel protein which is mainly expressed in astroglial foot processes. However, the pathogenetic role of AQP4 in NMO remains unknown. We herein report a typical case of NMO in which immunohistochemical analysis showed a lack of AQP4 in the spinal cord lesions. The loss of AQP4 was evident in the central gray matter, especially in the perivascular lesions where immunoglobulins and complements were deposited, and glial fibrillary acidic protein (GFAP) staining was weak in those lesions. However, GFAP was strongly stained at the reactive astrogliosis surrounding the lesions. Myelin basic protein (MBP)-stained myelinated fibers were relatively preserved in the lesions where AQP4 was lost. In contrast to these NMO lesions, AQP4 was expressed predominantly in the gray matter in control spinal cords, and AQP4 was preserved in demyelinating MS lesions. Our findings suggest that astrocytic impairment associated with humoral immunity against AQP4 may be primarily involved in the lesion formation of NMO, and that the pathomechanisms of NMO are different from those of MS in which demyelination is the primary pathology. ——— neuromyelitis optica; optic-spinal multiple sclerosis; aquaporin 4; demyelination; astroglia

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Neuromyelitis optica (NMO) has been considered an inflammatory demyelinating disease characterized by severe, often recurrent optic neuritis and transverse myelitis (Wingerchuk et al. 1999). Relapsing NMO has been called optic-spinal multiple sclerosis (OSMS) in Japan and other Asian countries (Misu et al. 2002) and this type of disease has been thought to be a subtype of multiple sclerosis (MS). The clinical features of NMO are female-predominance, negative oligoclonal IgG band, and longitudinally extensive and centrally located lesions in the spinal cord. Neuropathological studies in NMO showed that tissue necrosis with cavity formation and gray matter involvement were evident in addition to demyelination, and that vascular pathology including thickened vascular walls and hyalinization, and perivascular deposition of immunoglobulins and complements were unique pathological features in the lesions (Mandler et al. 1993; Lucchinetti et al. 2002; Misu et al. 2005). Those clinical, laboratory, MRI and histopathological features have been often used to delineate NMO as one variant of MS.

Recently, NMO-IgG, a serum autoantibody specific to NMO and OSMS, was found (Lennon et al. 2004). This NMO-IgG binds selectively to aquaporin-4 (AQP4) water channel (Lennon et al. 2005), a component of the dystroglycan protein complex located in astrocytic foot processes at the blood-brain-barrier (BBB). However, it remains unknown how AQP4 is involved in the pathogenesis of NMO.

Here we report a case of NMO in which an immunohistochemical study demonstrated a lack of AQP4 in the spinal cord lesions. This finding is unique to NMO but not to MS. We discuss the pathogenetic implications focusing on the distinction of NMO from MS.

CASE REPORT

Case summary of NMO

The patient had a total of five episodes of bilateral optic neuritis and six episodes of transverse myelitis in her 40s and 50s (We previously reported the details of the case [Nakamura et al. 2005]). The spinal cord MRI demonstrated a

longitudinally extending, cavitory lesion (C3-C7). The CSF examinations showed neutrophil-dominant pleocytosis, and oligoclonal band (OB) was negative. The patient's vision was severely impaired and she became wheel chair bound. At the age of 63, she died of acute respiratory failure. The consent of the patient's family was obtained, and autopsy was performed.

The neuropathological findings were as follows. The optic nerves and optic chiasma were severely atrophied. Microscopically, marked inflammation mainly consisting of neutrophils, macrophages, and severe tissue necrosis was seen in these lesions. Lymphocyte infiltration was scarce. In the cervical cord, there was an elongated cavitory lesion involving both the gray and white matters. Large numbers of neutrophils and macrophages were infiltrated in the lesions, while lymphocytes were few. Gram and periodic acid-Schiff stains for detecting bacteria and fungus were negative. There was an obsolete lesion with few inflammatory cell infiltrates extending from the cervical to the thoracic cord. In the lumbar cord, moderate inflammatory lesions were seen. There was an isolated cavitory lesion in the right frontal white matter, but there were no periventricular lesions typically seen in MS and there was no brainstem or cerebellar lesions. In the submandibular glands, inflammation consistent with a diagnosis of Sjögren's syndrome was not observed.

These clinical and neuropathological findings are typical of NMO.

Immunohistochemical study of AQP4, C9neo, astrocytes, and myelins

In the present case of NMO, we studied 8 sections of the medulla ($n = 2$), and the spinal cord at the cervical ($n = 3$), thoracic ($n = 2$), and lumbar ($n = 1$) levels, which included active lesions.

For immunostaining of AQP4, astrocytic protein (glial fibrillary acidic protein [GFAP]), activated complement (C9neo), and myelin proteins (myelin basic protein [MBP] and proteolipid protein [PLP]), we used the Avidin-Biotinylated enzyme Complex system or EnVision system

(Dako, Glostrup, Denmark). For double staining, we used horseradish peroxidase and alkaline phosphatase as enzymes, and diaminobenzidine hydrochloride or fuchsin as the chromogen. Selected sections were counterstained with a filtered solution of hematoxylin. Serial sections were simultaneously stained by isotype control. The following antibodies were used: anti-AQP4 antibody (Chemicon, Temecula, CA, USA), anti-GFAP antibody (6F2, Monosan, Uden, Netherlands), anti-MBP (N1546, Dako), anti-IgM (A0425, Dako), and anti-C9neo antibody (kindly provided from Dr. BP Morgan [Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff, UK]). Informed consent was obtained from the family of each patient prior to the study and this study was approved by the local ethics committee.

Immunohistochemical findings of NMO lesions

In the sections of a control subject's cervical cord (Fig. 1a), AQP4 was diffusely expressed in the whole cord, but the staining was stronger in the central gray matter than in the white matter. The fine structures corresponding to astrocytic foot processes and small blood vessels in the white matter were stained for AQP4 (Fig. 1b). There was no deposition of immunoglobulin or C9neo in the control subjects.

In our case of NMO (Fig. 2a), where there were extensive cavitory lesions in the central portion of the cervical cord, and the immunohistochemical expression of AQP4 (pink) was lacking in the lesions, although AQP4 was intensely stained in the surrounding areas where astrogliosis was seen. In a Luxol Fast Blue staining of the NMO lesions (Fig. 2b), there were cavitory and necrotic lesions at the peripheral region of a central gray matter lesion. A part of the lesion (encircled by a square in Fig. 2a) was studied in detail in serial sections by MBP (Fig. 2c), AQP4 (Fig. 2d), and GFAP (Fig. 2e). In contrast to the well-preserved MBP in the region (Fig. 2c), the immunohistochemical expression of AQP4 was completely lost at the severe, cavitory lesions (Fig. 2d) and the staining of GFAP in the lesions was faint (Fig. 2e), especially in the regions surrounding

the blood vessels. Meanwhile, astrogliosis strongly positive for AQP4 and GFAP was observed in the areas surrounding the lesions (Fig. 2d, 2e). At high-magnification of the perivascular area, enlarged blood vessels with thickened vessel walls could be seen (Fig. 3a-3d), which were much extremely larger than normal vessels (Fig. 1b). In contrast to the relatively preserved staining of MBP (Fig. 3a), AQP4 was virtually undetected in the tissues adjacent to the blood vessels (Fig. 3b). Perivascular astrocytes in those active lesions were swollen and were weakly positive for GFAP and observed as rosette like formations (Fig. 3c) coexisting with C9neo (Fig. 3d). In the double staining analysis (Fig. 4), AQP4 was completely lost in the areas surrounding a blood vessel with C9neo-positive active inflammation (Fig. 4a). These AQP4-free areas were encircled by the mesh-like staining of AQP4 (Fig. 4a), which may reflect the edematous and reactive astrogliosis. In the double-staining of IgM and GFAP (Fig. 4b), there were blood vessels in which IgM was deposited IgM-deposited in the center surrounded by GFAP-positive areas. Other cases of NMO had the same features as those seen in the present case.

Meanwhile, such an altered expression of AQP4 was not seen in any lesions of MS or in any area of the control spinal cord (data not shown). The staining pattern in the chronic lesions of MS was the exact opposite, that is, MBP staining was lost in the lesions, but the proliferation of GFAP- and AQP4-positive cells was seen, reflecting the demyelination associated with reactive astrogliosis (data not shown).

DISCUSSION

There have been long controversial whether NMO is a subtype of MS or a different disease. Recently, NMO-IgG was found in the serum specifically from patients with NMO, and its target antigen was identified as AQP4 water channel protein. However, the pathogenetic role of AQP4 in this disease remains unknown.

In the present report, we showed a lack of AQP4 in the spinal cord lesions of a case of NMO (Nakamura et al. 2005), that was one of the most

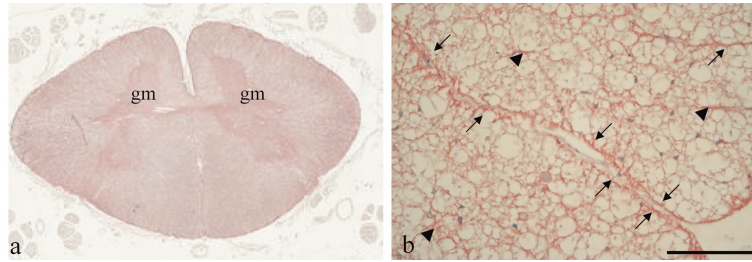


Fig. 1. Expression and distribution of aquaporin-4 (AQP4) in the normal spinal cord.
 a: The central gray matter (gm) was diffusely and highly positive for AQP4 (pink).
 b: In the white matter (high-magnification), fine structures corresponding to astrocytic foot processes (arrow heads), especially those surrounding small blood vessels in the white matter (arrows), were positive for AQP4. Scale bar represents 100 μm .

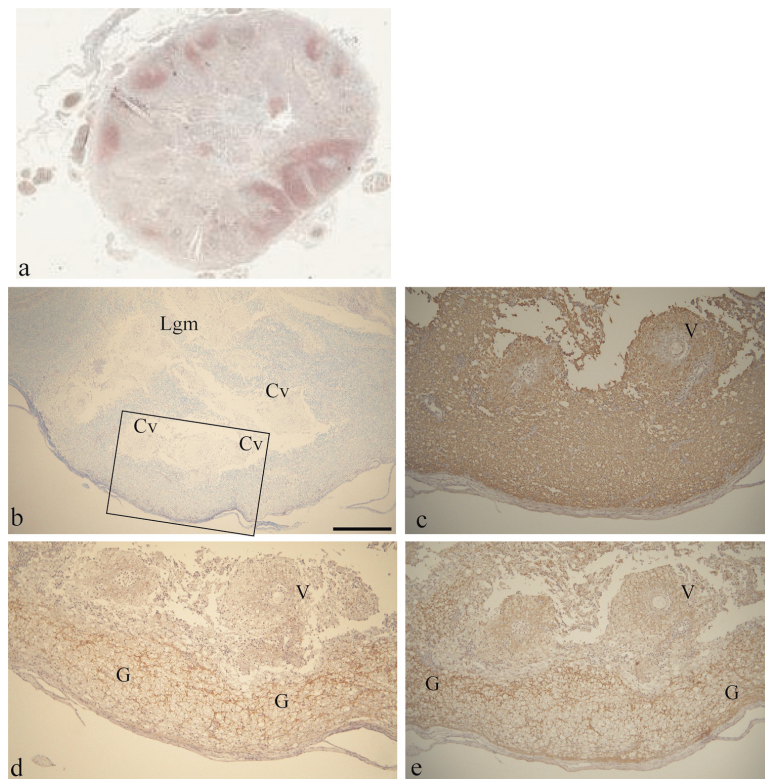


Fig. 2. Loss of aquaporin-4 (AQP4), and the staining of myelin and astrocytes in the spinal cord lesions of neuromyelitis optica (NMO).

a: In contrast to the normal spinal cord shown in Fig. 1a, there was no apparent expression of AQP4 in the central gray matter of NMO where tissue necrosis and cavity formation were seen, although some parts of the white matter were stained well (pink).

b: In the Luxol fast blue staining of a lesion edge, there were marked vascular proliferation, cavity formation (Cv) and necrosis. The area encircled by a square is the boundary of the extensive necrotic lesion in the gray matter (Lgm). Scale bar represents 200 μm .

Fig. 2c, 2d, and 2e are serial sections of the area encircled by a square in Fig. 2b. While the expression of myelin basic protein was relatively preserved (brown) (Fig. 2c), loss of AQP4 was evident in the lesion (Fig. 2d) and glial fibrillary acidic protein (GFAP) stained palely (Fig. 2e), especially in the regions surrounding the blood vessels (V). The areas outside of the lesion edge showed mesh-like, high expression of AQP4 and GFAP, reflecting reactive gliosis (G) (brown) (Fig. 2d and e).

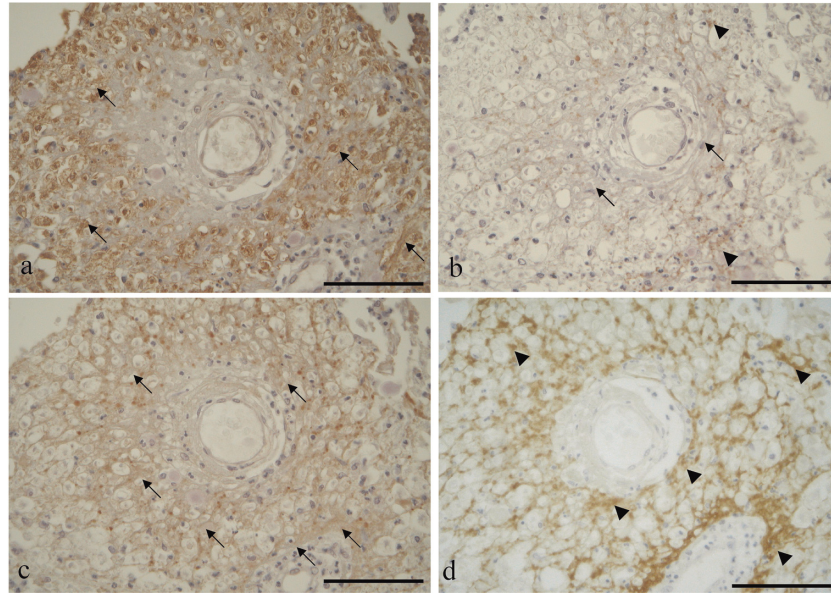


Fig. 3. Loss of Aquaporin-4 (AQP4), the staining of myelin and astrocytes, and deposition of complements at the active perivascular lesions of neuromyelitis optica (NMO).

Fig. 3a, 3b, 3c, and 3d are serial sections and higher magnifications of the perivascular region (V) of NMO in Fig 2c, 2d, and 2e. An enlarged blood vessel with a thickened vessel wall was seen in the center. In contrast to the relatively preserved staining of myelin basic protein (brown, arrows) (Fig. 3a), AQP4 was virtually undetected in the tissues adjacent to the enlarged blood vessel (arrows) except for debris-like stainings (arrow head) (Fig. 3b). Perivascular astrocytes were swollen and faintly positive for glial fibrillary acidic protein (arrows), and appeared as rosette-like formations (Fig. 3c) coexisting with activated complements (C9neo) (arrow heads) (Fig. 3d). Scale bar represents 100 μm .

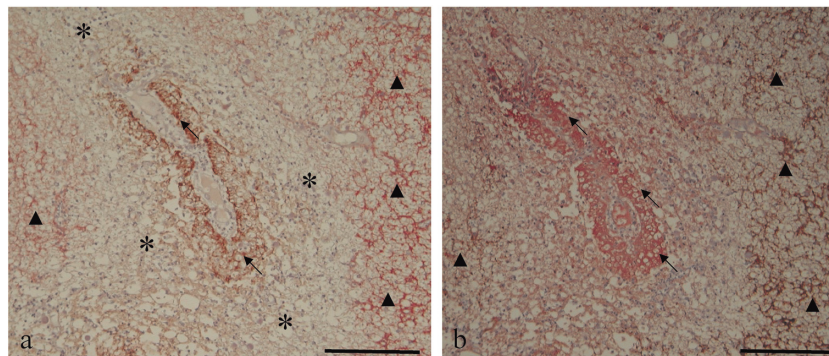


Fig. 4. Double-staining of aquaporin-4 (AQP4) and complements, and immunoglobulins and astrocytes at the active perivascular lesion in neuromyelitis optica.

a: There was a blood vessel with deposition of activated complements (C9neo) (brown, arrow) in the center surrounded by a mesh-like expression of AQP4 (pink, arrow heads). AQP4 was completely lacking in the region between the C9neo-deposited vessel and the mesh-like AQP4 expression (*).

b: There was a blood vessel with deposition of IgM (pink, arrows) in the center surrounded by glial fibrillary acidic protein (GFAP)-positive cells at the periphery of the Figure (brown, arrow heads). The staining of GFAP was also mesh-like as seen in AQP4. Scale bar represents 50 μm .

typical cases of the disease in terms of clinical and pathological features in our clinic. This lack of AQP4 in immunohistochemistry was observed in the lesions from other cases of NMO as well (data not shown), but it was never found in the lesions from MS cases.

In chronic MS lesions, astrogliosis in and around demyelinated plaques is one of most conspicuous findings and such astrocytic proliferation is considered a host response to inflammatory demyelination. Aoki-Yoshino et al. (2005) also showed an enhanced expression of AQP4 in MS brain lesions. That study demonstrated that astrocytes expressing AQP4 were more abundant at the periphery of demyelinated plaques as compared with the center of the lesion (Aoki-Yoshino et al. 2005). Another important finding in our case of NMO was the relatively preserved MBP-stained myelinated fibers in the lesions, even in the regions near the cavitory lesions where astrogliosis was usually impaired (Itoyama et al. 1985). These findings, especially a loss of AQP4 in the lesions, strongly suggest that NMO belongs to a distinct disease entity from MS.

AQP4 is rich in brain, especially in the gray matter of the spinal cord, and the periventricular and periaqueductal areas (Jung et al. 1994; Oshio et al. 2004), and more specifically this water channel protein is expressed in the astroglial foot processes at the BBB (Vizuete et al. 1999). AQP4 plays a crucial role in the control of the water balance in the brain, and its functional alteration is closely associated with brain edema (Jung et al. 1994; Vizuete et al. 1999; Verkman 2006). Since the perivascular deposition of immunoglobulins and activated complements was found in the lesions lacking AQP4, complement-dependent immunological processes, probably involving NMO-IgG binding to astrocytic AQP4, might cause degradation of the water channel protein and eventually lead to cytotoxic effects to astrocytes. The BBB is frequently damaged in NMO (Bergamaschi 2003), and thus NMO-IgG is likely to diffuse into the extravascular space and have direct contact to the AQP4 antigens on the astrocytic foot processes. The reason why transverse myelitis in NMO is often centrally located is

probably because the aberrant immunological reaction targets AQP4 which is predominantly present in the central gray matter of the spinal cord. Swelling of the cord is commonly seen in transverse myelitis of NMO, and the effects of NMO-IgG on AQP4 might also contribute to generating edema in the spinal cord. As shown in Figure (Fig. 3c and d), there were rosette-like formations of humoral factor depositions in the perivascular area, as described in a previous report (Lucchinetti et al. 2002), which may reflect the perivascular localization of the swollen astrocytic foot processes. Irregular mesh-like concentrated staining of AQP4 surrounding the regions lacking AQP4 (Fig. 4a) may reflect edematous change with reactive astrocytes, whose upregulation of AQP4 may lead to a more extensive autoimmune attack against AQP4.

We herein reported a case of NMO in which AQP4 water channel protein was lost in the active NMO lesions where immunoglobulins and complements were deposited. These changes were never seen in MS lesions. The findings strongly suggest that an immune reaction to AQP4 may be directly involved in the pathogenesis of NMO. The novel histopathological findings in our case of NMO require confirmation in larger-scale studies, and further analyses on the immunological attack on AQP4 in NMO are also needed.

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