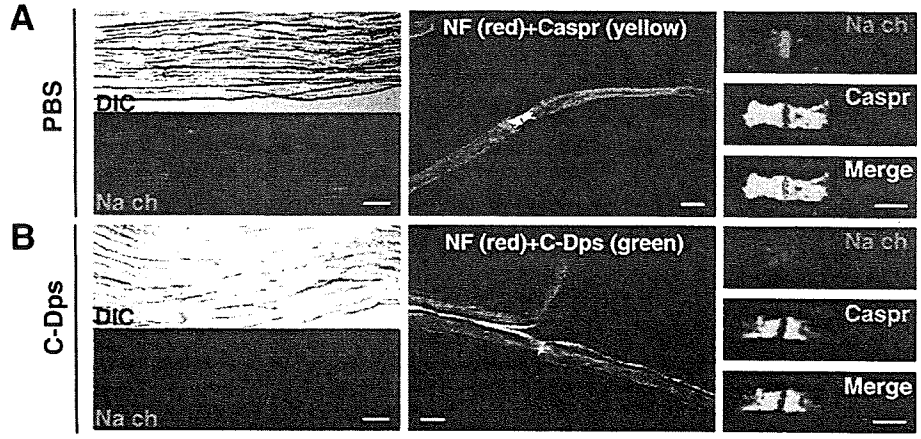

Fig. 2. C-Dps injected into the rat sciatic nerve induces down-modulation of nodal sodium channels. A. Immunostaining of PBS-injected nerves shows clustering of sodium channels in between Caspr at the nodes of Ranvier. Scattered sodium channel staining is seen throughout nerves. B. Immunostaining of C-Dps-injected nerves reveals the deposition of C-Dps on the nodes of Ranvier and on the outer surface of internodal myelin. In C-Dps-deposited nerves, unclustering and reduced expression of sodium channels are seen at the nodes of Ranvier. Scale bars in A and B, left panel = 50 μm, centre panel = 10 μm, and right panel = 5 μm. Caspr = contactin-associated protein, C-Dps = *Campylobacter jejuni* DNA-binding protein from starved cells, DIC = differential interference contrast, Na ch = sodium channel, NF = neurofilament, and PBS = phosphate-buffered saline.

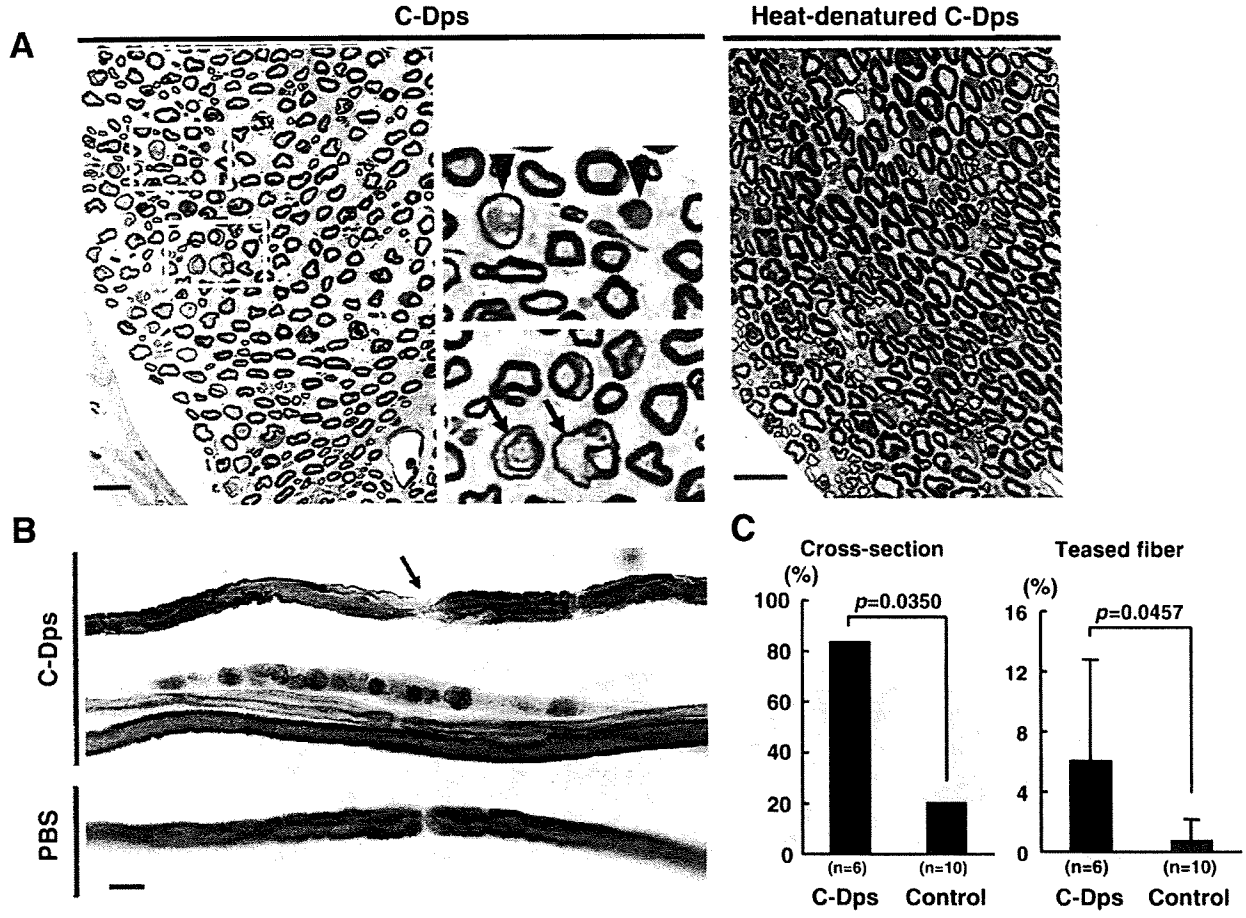

Fig. 3. C-Dps injected into the rat sciatic nerve induces paranodal demyelination and axonal degeneration. A. Epon-embedded sections of C-Dps-injected nerves showing a cluster of abnormal fibers exhibiting vesiculation of the myelin sheath (arrows), intramyelinic edema (arrows) and axonal degeneration (arrowhead), while heat-denatured C-Dps-injected fibers show no such changes. Insets = higher magnification. B. Teased fiber analysis clearly shows paranodal demyelination (arrows) and myelin ovoids. C. In each nerve, cross-sections and teased fibers around the injection sites were evaluated for the presence or absence of abnormal fiber changes. The frequency of affected nerves (vesiculation or axonal degeneration) in cross-sections is significantly higher in C-Dps-injected nerves ($n = 6$) than in the control group (PBS-injected; $n = 6$, heat-denatured C-Dps-injected; $n = 4$) (left panel). By teased fiber analysis, C-Dps-injected nerves show significantly higher percentages of abnormal fibers (paranodal demyelination and axonal degeneration) than control nerves (right panel). Scale bars in A = 20 μm, and B = 10 μm. C-Dps = *Campylobacter jejuni* DNA-binding protein from starved cells, and PBS = phosphate-buffered saline.

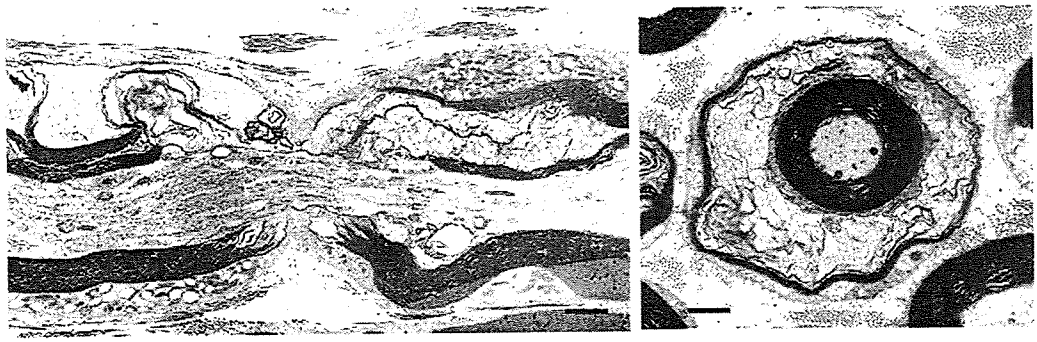

Fig. 4. Electron microscopy of longitudinal (left) and cross (right) sections of C-Dps-injected nerves. Electron microscopically, vesicular disruption of myelin is demonstrated only at the paranodes. Scale bar = 1 μm.

3.3. Electrophysiological effects of C-Dps injected into the rat sciatic nerve and applied to dissociated hippocampal neurons

Nerve conduction studies disclosed a rapid and significant reduction in CMAP amplitudes in C-Dps-injected sciatic nerves compared with pre-injection values after 10 to 420 min, while the motor conduction velocities (MCVs) did not change significantly (Fig. 5A,B). By contrast, no significant reduction in CMAP amplitudes or MCVs was noted following injection of PBS, heat-denatured C-Dps, or BSA. To address whether C-Dps directly affects the functions of voltage-gated sodium channels, we recorded Na^+ currents from dissociated hippocampal neurons, which express the same Nav 1.6 sodium channel as the nodes of Ranvier, using whole-cell recordings. Inward currents were blocked by the Na^+ channel blocker tetrodotoxin (TTX) at a concentration of 0.3 μM (data not shown). However, no significant change in TTX-sensitive inward currents could be seen following the application of C-Dps protein (5 μg/ml) (Fig. 5C). This result suggests that C-Dps does not alter sodium channel function.

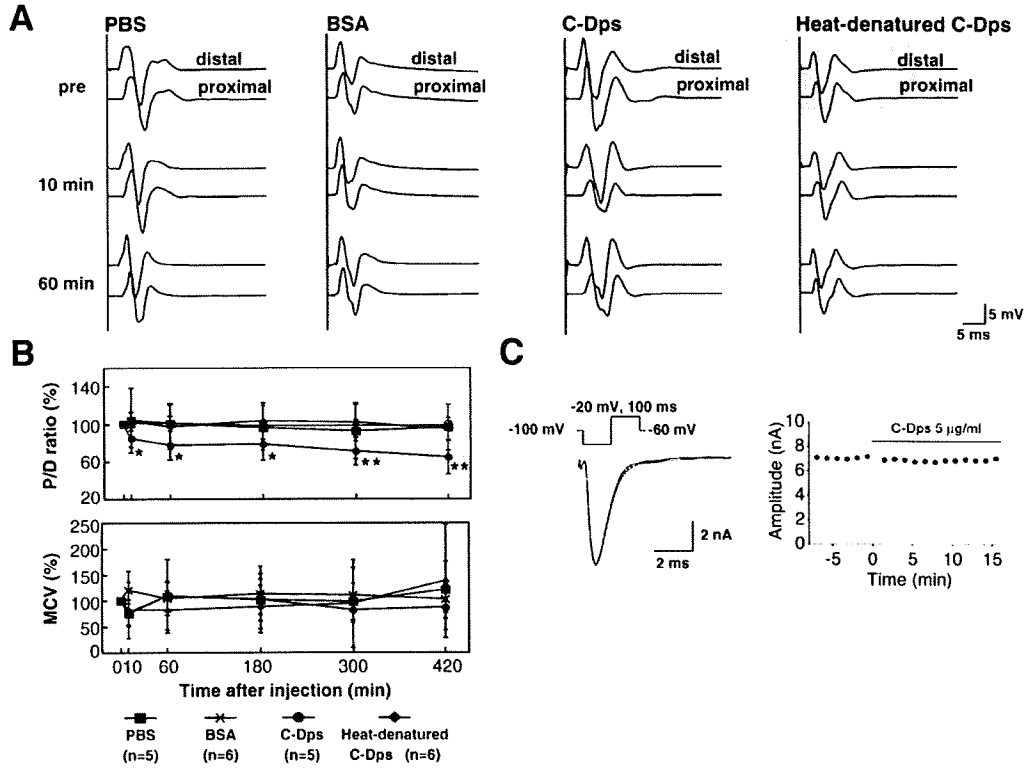

Fig. 5. C-Dps injected into the nerve induces a significant reduction in CMAP amplitudes. A. Sciatic nerves injected with PBS, BSA, C-Dps, or heat-denatured C-Dps were stimulated and recordings were obtained immediately before (pre) and 10 and 60 min after injection. A representative CMAP for each group is shown here. B. Mean values ± S.D. of proximal to distal amplitude ratios (P/D ratios) for the four groups (upper panel) are expressed as percent changes compared with the pre-treatment values in each group. At 10–420 min after injection, C-Dps-injected nerves show a significant decrease in the P/D ratio compared with the pre-injection values ($^*P<0.05$, $^{**}P<0.01$). PBS, BSA and heat-denatured C-Dps treatments do not induce significant reductions in CMAP amplitudes. Mean values ± S.D. of the MCVs of the four groups are expressed as percent changes compared with the pre-treatment values in each group (lower panel). No significant differences in MCVs are seen among any of the groups. C. C-Dps does not affect Na^+ currents in dissociated hippocampal neurons. (Left panel) Sample traces of evoked currents recorded before and 3 and 10 min after the start of the C-Dps application are superimposed and enlarged to show the fast inward currents. Pulse protocol: holding potential, −60 mV, 100 ms prepulse to −100 mV, and 100 ms test pulse to −20 mV. (Right panel) A plot of the inward current amplitude against time obtained from the same cell is shown in the left panel. The peak amplitude of the inward currents at 15 min after the start of the C-Dps applications was 102 ± 4 % of the control value ($n=4$, $P>0.05$). BSA = bovine serum albumin, C-Dps = *Campylobacter jejuni* DNA-binding protein from starved cells, MCV = motor conduction velocity, TTX = tetrodotoxin, and PBS = phosphate-buffered saline.

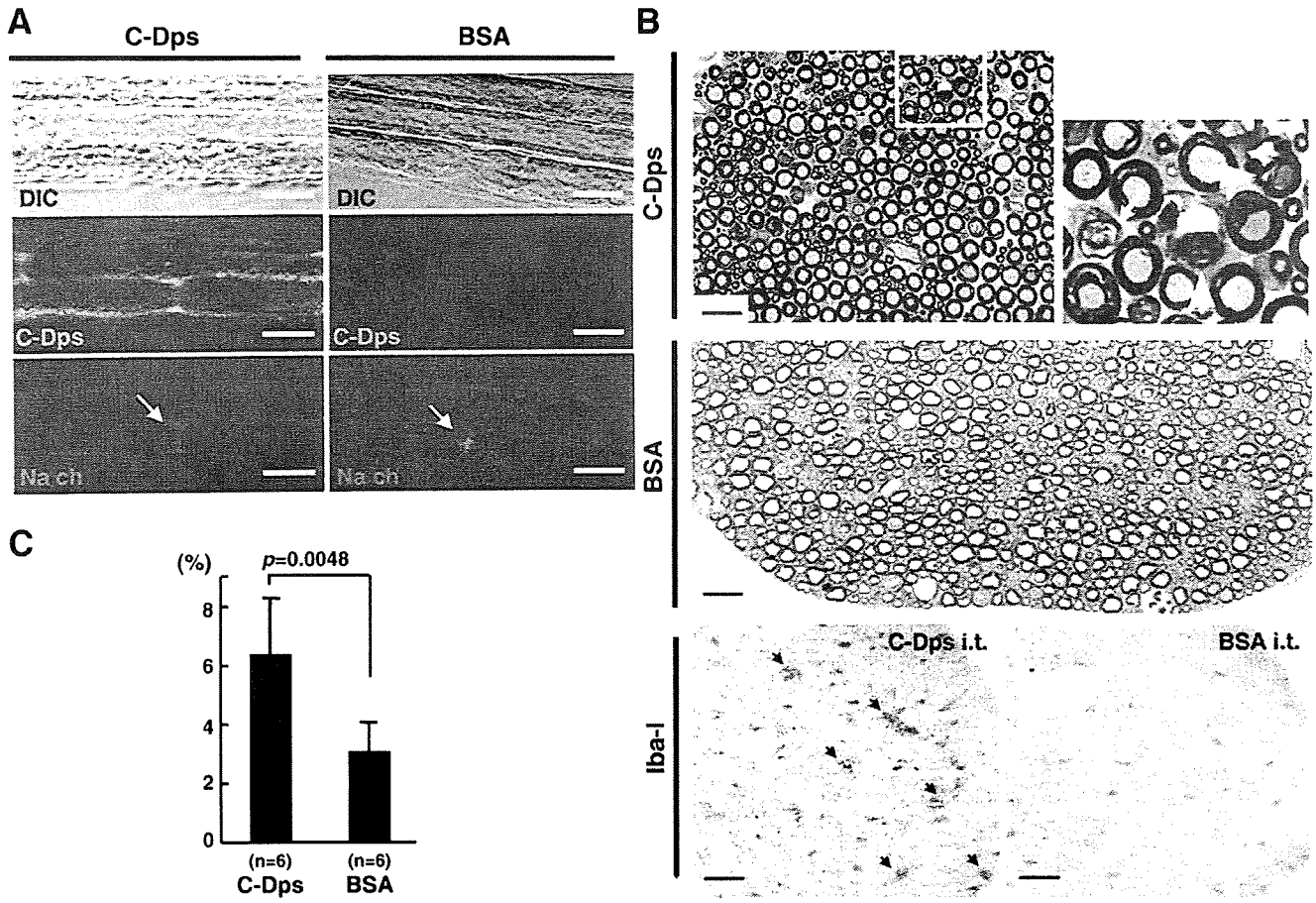

Fig. 6. Intrathecal infusion of C-Dps. A. In rats that received intrathecal infusion of C-Dps, the protein is found to be deposited in the outer parts of the myelin sheath and at the nodes of Ranvier in the cauda equina; reduced expression of sodium channels at the nodes of Ranvier is shown (arrow). B. Epon-embedded sections from C-Dps-infused rats show a cluster of abnormal fibers exhibiting vesiculation of the myelin sheath (arrow) and axonal degeneration (arrowhead), while no such changes are visible in sections from BSA-infused rats. Inset = higher magnification. Immunostaining with anti-Iba-1 antibody shows infiltration of Iba-1-positive cells in the cauda equina of C-Dps-infused rats (arrow), but not in those of BSA-infused rats. C. Within each nerve, the number of fibers showing abnormal changes was counted in cross-sections. The frequency of affected nerves (myelin vesiculation and/or axonal degeneration) is significantly higher in C-Dps-infused rats ($n=6$) than in BSA-infused rats ($n=6$). Scale bar in A = 10 μm, B = 20 μm. BSA = bovine serum albumin, C-Dps = *Campylobacter jejuni* DNA-binding protein from starved cells, DIC = differential interference contrast and Na ch = sodium channel.

3.4. Effects of intrathecally infused C-Dps

In rats subjected to the intrathecal infusion of C-Dps and intraperitoneal injection of LPS, C-Dps was found to be deposited in a scattered pattern in the cauda equina, especially in the outer part of the myelin sheath and the nodal region (Fig. 6A). Moreover, immunoreactivity for sodium channels was decreased in C-Dps-infused rats, but not in BSA-infused ones (Fig. 6A). In epon-embedded sections, vesiculation of the myelin sheath and axonal degeneration were observed in C-Dps-infused rats, but not in BSA-infused ones (Fig. 6B). In addition, Iba-1-positive macrophages were observed around the affected nerve fibers of the cauda equina in C-Dps-treated rats, but not in BSA-treated ones (Fig. 6B). The frequencies of affected nerves showing such changes (myelin vesiculation and/or axonal degeneration) were significantly higher in the former group of rats than in the latter (6.36% vs. 3.05%, $P=0.0048$) (Fig. 6C).

4. Discussion

The present study is the first to show that Dps protein from *C. jejuni* induces rapid paranodal myelin detachment and down-modulation of nodal sodium channels by binding to sulfatide on the outermost parts of myelin and the nodes of Ranvier *in vivo*. Because there was no significant change in the level of internodal myelin, and expression of Caspr, a marker of paranodal junctions between the axonal membrane and terminal loop of myelinated glial cells derived from the axonal membrane, was also impaired in C-Dps-injected nerves, it is possible that the primary effect of C-Dps is paranodal myelin detachment. Such paranodal myelin detachment could result in a massive leak of driving currents, and thereby block nerve conduction [16]. The rapid reduction of CMAP amplitudes observed in C-Dps-injected nerves may in part be explained by such paranodal myelin detachment induced by C-Dps.

Paranodal demyelination is considered to be the earliest and mildest change observed in humans and chickens with *C. jejuni*-related GBS, either spontaneously or experimentally induced [2,17]. This change has never been reproduced in a rabbit model using immunization with either brain gangliosides or purified GM1, even in the presence of high titers of anti-GM1 antibodies [18,19]. Although the alteration of nodal sodium channels has never been studied immunohistochemically in patients with *C. jejuni*-related GBS, blockade of sodium channels has been electrophysiologically indicated to be one of the earliest changes in this disease [20,21]. This accounts in part for the reduction in CMAP amplitudes in the human disease [21–23]. The apparent lack of pathology in some severely paralyzed humans [24] and chicken models [17] may be explained by selective down-modulation of nodal sodium channels inducing a conduction block without evident internodal demyelination. Axonal degeneration associated with paranodal myelin detachment was also observed in both C-Dps-injected nerves and C-Dps-infused cauda equina.

in good accord with the axonal pathology seen in patients with *C. jejuni*-related GBS.

In experimentally infected chickens, diarrhea begins 2–4 days after inoculation and lasts for up to 14 days, while the earliest signs of paralysis appear 5 days after inoculation [17]. Because of severe paralysis in the absence of apparent morphological changes and inflammation, the possible existence of a neurotoxin produced by the organism was suggested [17]. C-Dps, which is liberated by bacterial cell lysis during the diarrhea period, may thus be a candidate neurotoxin produced by this organism, possibly explaining the early changes in the nodal and paranodal regions without inflammation.

There are several possible mechanisms by which C-Dps could induce down-modulation of nodal sodium channels. First, cerebroside sulfotransferase-knock-out mice, which have a selective sulfatide deficiency, show disruption of paranodes without demyelination of internodal myelin [9,10]. In this model, sodium channels cluster normally during development, but clustering decreases with age leading to disorganization of the paranodal structures, and sulfatide is considered to be essential for the maintenance of paranodes [9,10]. It is thus possible that C-Dps interferes with the functions of paranodal sulfatide, resulting in myelin detachment and unclustering of sodium channels. Down-modulation of paranodal Caspr in C-Dps-injected nerves also supports this possibility. Second, sulfatide is essential for anchoring the basal lamina to the Schwann cell abaxonal membrane through binding to laminin-1 and -2 [25]. Disruption of the anchoring between the basal lamina and Schwann cells can be achieved by the selective deletion of Schwann cell dystroglycan [26]. Thus C-Dps may also induce down-modulation of nodal sodium channels by interfering with the interaction between laminin-2 on the basal lamina and sulfatide on the Schwann cell abaxonal membrane. Perturbation of peripheral nerve sulfatide functions may cause axonal changes in a fashion similar to anti-sulfatide antibody-associated neuropathy, in which axonal degeneration together with demyelination has been reported [11,12,27,28]. Further studies are required to elucidate the precise mechanism by which C-Dps affects the axo-glial interface. Last, considering the rapid reduction in CMAP amplitudes induced by intraneural injection of C-Dps, we tested the direct effect of C-Dps on Na^+ currents in dissociated hippocampal neurons expressing the same Nav1.6 sodium channel molecule as peripheral nerves; however, no direct effect was noted.

With anti-GM1 antibody treatment in either isolated nerve preparations or following intraneural injection, a conduction block has been observed in some studies [29,30], but not in others [5,31,32], in part because of the inaccessibility of the antigen to normal nerves [4,29]. C-Dps-induced paranodal changes in the early phase may allow the anti-GM1 antibody to access target antigens at the node [4], and in conjunction with complements, anti-GM1 antibody may cause persistent axonal damage [4,29,30,33] in the late phase when the antibody titer reaches its peak [34]. Thus, C-Dps-induced nodal damage and anti-GM1 antibody-mediated axonal injury are not mutually exclusive, but they may act in concert to induce the axonal pathology seen in patients with *C. jejuni*-related GBS.

Finally, to produce myelinated nerve fiber damage, both C-Dps and the anti-GM1 antibody must enter into the nerve through the BNB. *C. jejuni* penetrates the intestinal epithelial barrier [35] and induces expression of proinflammatory cytokines, such as TNF-α, IL-1β, IFN-γ, IL-6 and IL-8, in dendritic cells [36] and monocytes [37]. Among these, TNF-α, the levels of which have been shown to be elevated in the sera of GBS patients [38], can disrupt the BNB [14]. Moreover, *C. jejuni* harbors LPS on the outer membrane [1], and this bacterial product also renders the BNB leaky [39].

In summary, we successfully reproduced paranodal myelin detachment and axonal degeneration, both of which are key features of *C. jejuni*-related GBS, by intraneural injection as well as intrathecal infusion of C-Dps. Moreover, we showed the occurrence of rapid sodium channel down-modulation at the node following C-Dps treatment, which may explain the conduction block seen in this condition. Therefore, we hypothesize that, during the diarrhea period, C-Dps liberated by *C. jejuni* enters into the blood stream, gets across the weakened BNB with the assistance of LPS and various proinflammatory cytokines, binds to the paranodes, interferes with paranodal sulfatide function and causes axonal damage, resulting in paranodal myelin detachment and loss of nodal sodium channels. Anti-GM1 antibodies are likely to play a major role in producing nodal membrane damage by complement activation and causing the disruption of sodium channel clusters [32] once nodal GM1 is exposed following paranodal structure disruption. Moreover, Usuki et al [40] recently reported that anti-lipid A antibody from *C. jejuni*-infected individuals also down-modulates sodium channels in cultured neuronal cells. Therefore, in addition to these antibodies against gangliosides and lipids, C-Dps may be a co-factor underlying the axonal pathology in *C. jejuni*-related GBS patients through paranodal structure disruption. However, there is a time lag of a few days between the diarrhea period when C-Dps is produced and the appearance of anti-ganglioside antibody. Thus, the mechanism of the synergistic effects of these two components would be a subject for an important future study. In addition, the presence of C-Dps does not explain GBS following other infections in which anti-GM1 antibodies also occur; however, because some bacteria contain similar proteins belonging to the Dps protein family, it may be worth investigating such bacterial proteins that bind to human peripheral nerves.

Acknowledgments

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Neuroimmunological Disease Research Committee grant from the Ministry of Health, Labour and Welfare of Japan and a grant for Research on Brain Science. We thank Tomo Iwashima and Magnus Hallstrom for their technical assistance.

References

[1] Yuki N. Infectious origins of, and molecular mimicry in, Guillain–Barré and Fisher syndromes. Lancet Infect Dis 2001;1:29–37.
[2] Griffin JW, Li CY, Macko C, Ho TW, Hsieh ST, Xue P, et al. Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain–Barré syndrome. J Neurocytol 1996;25:33–51.
[3] Ariga T, Yu RK. Antiglycolipid antibodies in Guillain–Barré syndrome and related diseases: review of clinical features and antibody specificities. J Neurosci Res 2005;80:1–17.
[4] Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. Brain 2002;125:2591–625.
[5] Hirota N, Kaji R, Bostock H, Shindo K, Kawasaki T, Mizutani K, et al. The physiological effect of anti-GM1 antibodies on saltatory conduction and transmembrane currents in single motor axons. Brain 1997;120:2159–69.
[6] Sheikh KA, Zhang G, Gong Y, Schnaar RL, Griffin JW. An anti-ganglioside antibody-secreting hybridoma induces neuropathy in mice. Ann Neurol 2004;56:228–39.
[7] Ishikawa T, Mizunoe Y, Kawabata S, Takade A, Harada M, Wai SH, et al. The iron-binding protein Dps confers hydrogen peroxide stress resistance to *Campylobacter jejuni*. J Bacteriol 2003;185:1010–7.
[8] Teneberg S, Miller-Podraza H, Lampert HC, Evans Jr DJ, Evans DG, Danielsson D, et al. Carbohydrate binding specificity of the neutrophil-activating protein of *Helicobacter pylori*. J Biol Chem 1997;272:19067–71.
[9] Honke K, Hirahara Y, Dupree J, Suzuki K, Popko B, Fukushima K, et al. Paranodal junction formation and spermatogenesis require sulfoglycolipids. Proc Natl Acad Sci USA 2002;99:4227–32.
[10] Ishibashi T, Dupree JL, Ikenaka K, Hirahara Y, Honke K, Peles E, et al. A myelin galactolipid, sulfatide, is essential for maintenance of ion channels on myelinated axon but not essential for initial cluster formation. J Neurosci 2002;22:6507–14.
[11] Carpo M, Meucchi N, Allaria S, Marmiroli P, Monaco S, Toscano A, et al. Anti-sulfatide IgM antibodies in peripheral neuropathy. J Neurol Sci 2000;176:144–50.
[12] Erb S, Ferracin F, Fuhr P, Rosler KM, Hess CW, Kuntzer T, et al. Polyneuropathy attributes: a comparison between patients with anti-MAG and anti-sulfatide antibodies. J Neurol 2000;247:767–72.
[13] Nardelli E, Bassi A, Mazzi G, Anzini P, Rizzuto N. Systemic passive transfer studies using IgM monoclonal antibodies to sulfatide. J Neuroimmunol 1995;63:29–37.
[14] Uncini A, Di Muzio A, Di Guglielmo G, De Angelis MV, De Luca G, Lugaresi A, et al. Effect of rhTNF-alpha injection into rat sciatic nerve. J Neuroimmunol 1999;94:88–94.
[15] Mizoguchi Y, Ishibashi H, Nabekura J. The action of BDNF on GABAA currents changes from potentiating to suppressing during maturation of rat hippocampal CA1 pyramidal neurons. J Physiol 2003;548:703–9.

[16] Yuki N, Kuwabara S. Axonal Guillain–Barre syndrome: carbohydrate mimicry and pathophysiology. J Perihperal Nerv Sys 2007;12:238–49.
[17] Li CY, Xue P, Tian WQ, Liu RC, Yang C. Experimental *Campylobacter jejuni* infection in the chicken: an animal model of axonal Guillain–Barré syndrome. J Neurol Neurosurg Psychiatry 1996;61:279–84.
[18] Yuki N, Yamada M, Koga M, Odaka M, Susuki K, Tagawa Y, et al. Animal model of axonal Guillain–Barré syndrome induced by sensitization with GM1 ganglioside. Ann Neurol 2001;49:712–20.
[19] Susuki K, Nishimoto Y, Yamada M, Baba M, Ueda S, Hirata K, et al. Acute motor axonal neuropathy rabbit model: immune attack on nerve root axons. Ann Neurol 2003;54:383–8.
[20] Kuwabara S, Ogawara K, Sung JY, Mori M, Kanai K, Hattori T, et al. Differences in membrane properties of axonal and demyelinating Guillain–Barré syndromes. Ann Neurol 2002;52:180–7.
[21] Kuwabara S, Ogawara K, Misawa S, Koga M, Mori M, Hiraga A, et al. Does *Campylobacter jejuni* infection elicit "demyelinating" Guillain–Barré syndrome? Neurology 2004;63:529–33.
[22] Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, et al. Guillain–Barré syndrome in northern China. Relationship to *Campylobacter jejuni* infection and anti-glycolipid antibodies. Brain 1995;118:597–605.
[23] McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, et al. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol 1993;33:333–42.
[24] Griffin JW, Li CY, Ho TW, Xue P, Macko C, Gao CY, et al. Guillain–Barré syndrome in northern China. The spectrum of neuropathological changes in clinically defined cases. Brain 1995;118:577–95.
[25] Li S, Liquari P, McKee KK, Harrison D, Patel R, Lee S, et al. Laminin-sulfatide binding initiates basement membrane assembly and enables receptor signaling in Schwann cells and fibroblasts. J Cell Biol 2005;169:179–89.
[26] Saito F, Moore SA, Barresi R, Henry MD, Messing A, Ross-Barta SE, et al. Unique role of dystroglycan in peripheral nerve myelination, nodal structure, and sodium channel stabilization. Neuron 2003;38:747–58.
[27] Lopate G, Parks BJ, Goldstein JM, Yee WC, Friesenhahn GM, Pestronk A. Polyneuropathies associated with high titre antisulfatide antibodies: characteristics of patients with and without serum monoclonal proteins. J Neurol Neurosurg Psychiatry 1997;62:581–5.
[28] Dabby R, Weimer LH, Hays AP, Olarte M, Latov N. Antisulfatide antibodies in neuropathy: clinical and electrophysiologic correlates. Neurology 2000;54:1448–52.
[29] Willison HJ, O'Hanlon G, Paterson G, O'Leary CP, Veitch J, Wilson G, et al. Mechanisms of action of anti-GM_1 and anti-GQ_{1b} ganglioside antibodies in Guillain–Barré syndrome. J Infect Dis 1997;176:S144–9.
[30] Takigawa T, Yasuda H, Kikkawa R, Shigeta Y, Saida T, Kitasato H. Antibodies against GM_1 ganglioside affect K^+ and Na^+ currents in isolated rat myelinated nerve fibers. Ann Neurol 1995;37:436–42.
[31] Buchwald B, Toyka KV, Zielasek J, Weishaupt A, Schweiger S, Dudel J. Neuromuscular blockade by IgG antibodies from patients with Guillain–Barré syndrome: a macro-patch-clamp study. Ann Neurol 1998;44:913–22.
[32] Dilley A, Gregson NA, Hadden RDM, Smith KJ. Effects on axonal conduction of anti-ganglioside sera and sera from patients with Guillain–Barré syndrome. J Neuroimmunol 2003;139:133–40.
[33] Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci 2007;27:3956–67.
[34] Press R, Mata S, Lolli F, Zhu J, Andersson T, Link H. Temporal profile of anti-ganglioside antibodies and their relation to clinical parameters and treatment in Guillain–Barré syndrome. J Neurol Sci 2001;190:41–7.
[35] van Spreeuwel JP, Duursma GC, Meijer CJ, Bax R, Rosekrans PC, Lindeman J. *Campylobacter* colitis: histological immunohistochemical and ultrastructural findings. Gut 1985;26:945–51.
[36] Hu L, Bray MD, Osorio M, Kopecko DJ. *Campylobacter jejuni* induces maturation and cytokine production in human dendritic cells. Infect Immun 2006;74:2697–705.
[37] Jones MA, Tötemeyer S, Maskel DJ, Bryant CE, Barrow PA. Induction of proinflammatory responses in the human monocytic cell line THP-1 by *Campylobacter jejuni*. Infect Immun 2003;71:2626–33.
[38] Sharief MK, McLean B, Thompson EJ. Elevated serum levels of tumor necrosis factor-alpha in Guillain–Barré syndrome. Ann Neurol 1993;33:591–6.
[39] Xaio H, Banks WA, Niehoff ML, Morley JE. Effect of LPS on the permeability of the blood–brain barrier to insulin. Brain Res 2001;896:36–42.
[40] Usuki S, Nakatani Y, Taguchi K, Fujita T, Tanabe S, Ustunomiya I, et al. Topology and patch-clamp analysis of the sodium channel in relationship to the anti-lipid A antibody in *Campylobacteriosis*. J Neurosci Res 2008;86:3359–74.

Longitudinally extensive myelopathy in Caucasians: a West Australian study of 26 cases from the Perth Demyelinating Diseases Database

W Qiu, J-S Wu, M-N Zhang, et al.

J Neurol Neurosurg Psychiatry 2010 81: 209-212 originally published online August 25, 2009
doi: 10.1136/jnnp.2009.172973

Longitudinally extensive myelopathy in Caucasians: a West Australian study of 26 cases from the Perth Demyelinating Diseases Database

W Qiu,[1] J-S Wu,[1] M-N Zhang,[2] T Matsushita,[3] J-i Kira,[3] W M Carroll,[1] F L Mastaglia,[1] A G Kermode[1]

[1]Centre for Neuromuscular and Neurological disorders, University of Western Australia, and Department of Neurology, Sir Charles Gairdner Hospital, Queen Elizabeth II Medical Centre, Perth, Western Australia
[2]First Hospital of Shanxi Medical University, Taiyuan, PR China
[3]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence to
Professor A G Kermode, Australian Neuromuscular Research Institute, Sir Charles Gairdner Hospital; Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Queen Elizabeth II Medical Centre, 6009, Perth, WA, Australia;
kermode@me.com

WQ and J-SW contributed equally to this manuscript

Received 2 February 2009
Revised 18 May 2009
Accepted 29 June 2009
Published Online First 25 August 2009

ABSTRACT

Objectives To characterise West Australian cases of longitudinally extensive myelopathy (LEM).

Methods Twenty six patients with LEM were identified from a cohort of 983 patients with demyelinating disease. Clinical and MRI data and AQP4-IgG results were reviewed.

Results LEM cases were classified as conventional MS (CMS) 13, neuromyelitis optica (NMO) 7, and isolated LEM 6. LEM was the initial presentation in 13/26 cases. In CMS cases lesions were mainly in the lower cervical cord (C4–C7) whereas in NMO and isolated LEM they were more often thoracic and were longer. The severity of disability was highly variable but was greater in the NMO than the CMS group. Only one of 20 patients tested was seropositive for AQP4-IgG.

Conclusion LEM occurred as part of CMS or NMO or in isolation. Patients with LEM had highly heterogeneous clinical characteristics and a low rate of AQP4-IgG seropositivity.

Longitudinally extensive myelopathy (LEM) is defined as myelopathy with cord lesions extending over three or more vertebral levels on sagittal spinal MRI.[1–5] Although LEM has been regarded as characteristic of NMO and Asian optic-spinal multiple sclerosis (OSMS),[1 5] it also occurs in conventional MS (CMS), both in Asian and Caucasian populations.[4–7] Few studies on the clinical profile of LEM in Caucasian populations have been reported and there have been no previous studies from the Southern Hemisphere.

In this study we investigated the frequency, clinical profile, MRI findings and frequency of AQP4-IgG antibody in LEM cases from the Perth Demyelinating Diseases Database (PDDD).

METHODS

Patients

A group of 660 cases with spinal cord MRI were selected from a larger cohort of 983 patients with demyelinating disease in the PDDD: the diagnoses were CMS 574, NMO 27, isolated myelopathy 45, optic neuritis 6 and atypical demyelinating disease 8. After reviewing the spinal MRI studies 26 patients with LEM were identified. The following clinical parameters were analysed: age, gender, onset features, disease duration, relapse number and relapse rate, and last Extended Disability Status Scale (EDSS) score. EDSS scores were divided into three ranges: 0–3, mild disability; 3.5–5.5, moderate disability; 6.0–10, severe disability.

MRI

All 26 patients had cerebral and spinal MRI on one or more occasions on a 1.5 or 3.0 T scanner. T1 with and without gadolinium enhancement, T2, fluid attenuated inversion recovery (FLAIR) and short tau inversion recovery (STIR) sequences were performed in most cases. Spinal MRI was performed within 4 weeks of the onset of myelopathic symptoms. Lesions on spinal MRI were analysed for anatomical location (sagittal and axial) in the cord, length and the presence of cord swelling and gadolinium enhancement.

Aquaporin-4 antibody

The AQP4-IgG assay was performed blinded by Dr Takuya Matsushita at Kyushu University by immunofluorescence assay using green fluorescent protein (GFP)-AQP4 fusion protein transfected HEK-293T cells as described previously.[8] Sera were taken during a clinical relapse in five patients and during remission in 15 patients.

Statistics

Statistical analyses of age-at-onset and at examination were performed using an analysis of the Student t test. Comparisons of relapse number, disease duration, relapse rate, EDSS score and length of MRI spinal cord lesions were performed using the Mann–Whitney U test between groups. Differences in gender, OCB positivity and MRI features between groups were tested by Fisher exact test. All statistical analyses were performed using the SPSS V16.0 for Mac. p Values <0.05 were considered statistically significant.

This study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee, and informed consent was obtained from all study participants.

RESULTS

Demographic and clinical features

As shown in table 1, there were 15 females and 11 males and all were Caucasian. Thirteen patients were classified as CMS, seven as NMO, and six as isolated LEM (one monophasic and five recurrent). All CMS patients fulfilled the 2001 McDonald criteria for definite MS and had a relapsing-remitting course.[2] Cases classified as NMO all fulfilled the criteria of Wingerchuk *et al* 1999, and all but one had recurrent episodes.[3] All isolated LEM cases had normal VEPs and an extensive workup, to exclude other specific causes, was negative except

Table 1 Summary of clinical, OCB, antibody against aquaporin-4 (AQP4-IgG) and spinal MRI characteristics* data of 26 patients with longitudinal extensive myelopathy

		Non-conventional multiple sclerosis		
	Conventional multiple sclerosis (n = 13)	Total (n = 13)	Neuromyelitis optica (n = 7)	Isolated longitudinal extensive myelopathy (n = 6)
Sex ratio (male/female)	4/9	7/6	4/3	3/3
Age at examination (years)	44.9 (11.3)	51.8 (12.2)	55.9 (13.1)	47.0 (10.3)
Age at onset (years)	30.6 (8.0)	43.6 (13.0)†	46.0 (14.2)†	40.2 (13.8)†
Disease duration (years)	14.3 (10.0)	8.2 (7.4)	9.9 (9.7)	6.3 (3.3)
Relapse number	4.4 (2.5)	2.8 (1.5)	3.4 (1.5)	2.2 (1.2)
Relapse rate	0.6 (0.7)	0.6 (0.5)	0.8 (0.6)	0.4 (0.3)
Final Extended Disability Status Scale	5.1 (2.4)	6.4 (3.6)	8.0 (2.9)†	5.2 (3.7)
Oligoclonal band in cerebrospinal fluid (+), n (%)	3/5 (60%)	2/10 (20%)	0/6 (0%)	2/4 (50%)
AQP4-IgG (+), n (%)	0/10 (0%)	1/10 (10%)	0/4 (0%)	1/6 (16.7%)
Spinal cord lesion length, segments (range)	4.5 (1.3) (3 to 7)	8.5 (4.5)† (3 to 18)	7.1 (3.2) (3 to 12)	10.2 (5.5)† (4 to 18)
Sagittal distribution, n (%)				
C1–C3	6 (46.2%)	5 (38.5%)	3 (42.9%)	2 (33.3%)
C4–C7	10 (76.9%)	7 (53.8%)	4 (57.1%)	3 (50.0%)
T1–T4	1 (7.7%)	8 (61.5%)†	4 (57.1%)†	4 (66.7%)†
T5–T8	4 (30.8%)	7 (53.8%)	2 (28.6%)	5 (83.3%)
T9–T12	0 (0%)	3 (23.1%)	2 (28.6%)	1 (16.7%)
Axial distribution, n (%)				
Holocord‡	3 (23.1%)	4 (30.8%)	2 (28.6%)	2 (33.3%)
Central cord	6 (46.1%)	7 (53.8%)	3 (42.9%)	4 (66.7%)
Peripheral cord	4 (30.8%)	2 (15.4%)	2 (28.6%)	0 (0%)
Swelling cord, n (%)	5 (38.5%)	10 (76.9%)	6 (85.7%)	4 (66.7%)
Gadolinium enhancement, n (%)	2/8 (25%)	9/12 (75.0%)	6/7 (85.7%)	3/5 (60%)

*Only longitudinal extensive myelopathy lesions were analysed; short lesions were not included.
†Statistically significant in comparison with conventional multiple sclerosis ($p<0.05$).
‡Holocord applies to total transverse cord involvement.

for one patient with an elevated anti-nuclear antibody titre (1:320).

The average onset age in the whole group was 37.1 years (18–63 years) and was significantly lower in the CMS group (30.6; $p<0.05$). Average disease duration in the whole group was 11.3 years (range 0–32 years): 14 patients >10 years, 9>15 years and 5>20 years. Six of the 26 patients were followed up from disease onset to death after mean periods of 1–21 years (mean 6.8 years).

The severity of disability was variable but was greater in the NMO group: mean last EDSS was 8.0 in the NMO group and 5.1 in the CMS group ($p<0.05$). Six of the seven NMO patients had a final EDSS score ≥6.0. Eight patients were classified in the mild disability group, three in the moderate disability group and 15 in the severe disability group. Two patients had a benign course with an EDSS score <3.0 more than 10 years after the onset of the LEM.

LEM was the initial presentation in 13 of the 26 patients: of these one had monophasic disease, while the remaining 12 relapsing cases were classified as CMS (4), NMO (3) and recurrent LEM (5). In the other 13 cases (nine CMS, four NMO) LEM occurred after intervals of 1–27 years following the initial presentation.

MRI findings

The extent and location of spinal cord MRI lesions are summarised in table 1. The mean lesion length was longer in the NMO group than in CMS group: 7.1 (range 3–12 segments) versus 4.5 (range 3–7 segments) and was longest in the recurrent LEM cases: 10.2 (range 4–18 segments) ($p<0.05$). Extremely long lesions spanning more than 10 vertebral segments were seen in two NMO and two recurrent LEM cases (figure 1). Lesions in CMS were mostly located in the lower cervical cord (C4–C7), but only rarely in the upper thoracic cord (T1–T4), which was significantly different from the NMO and recurrent LEM cases ($p<0.05$) in which thoracic lesions were more common. On axial MRI LEM lesions occupied the centre of the cord or the whole cord in all six isolated LEM cases, 5/7 NMO cases and 9/13 CMS cases. Cord swelling and enhancement were more common in the NMO and recurrent LEM groups.

All except one of the CMS patients had typical cerebral lesions fulfilling the Barkhof criteria.[2] Of the NMO patients, one had a medullary lesion, and three with normal MRIs at onset later developed cerebral lesions, one a tumefactive lesion and the others typical MS lesions. Of the six isolated LEM patients, one developed tumefactive lesions in the left occipital and temporal lobes 2 years after onset, a brain biopsy confirmed the diagnosis of demyelinating disease, and another developed a medullary lesion.

Aquaporin-4 antibody

AQP4-IgG was tested in 20 patients (four patients were deceased, and in two patients samples could not be obtained): 10 CMS, four NMO and six isolated LEM. Only one (relapsing LEM) was seropositive (titre 1:16).

DISCUSSION

As in previous studies, LEM was not confined to cases of NMO or isolated myelopathy but also occurred in CMS with a frequency of 2.3%. This is similar to the figure of 3% reported

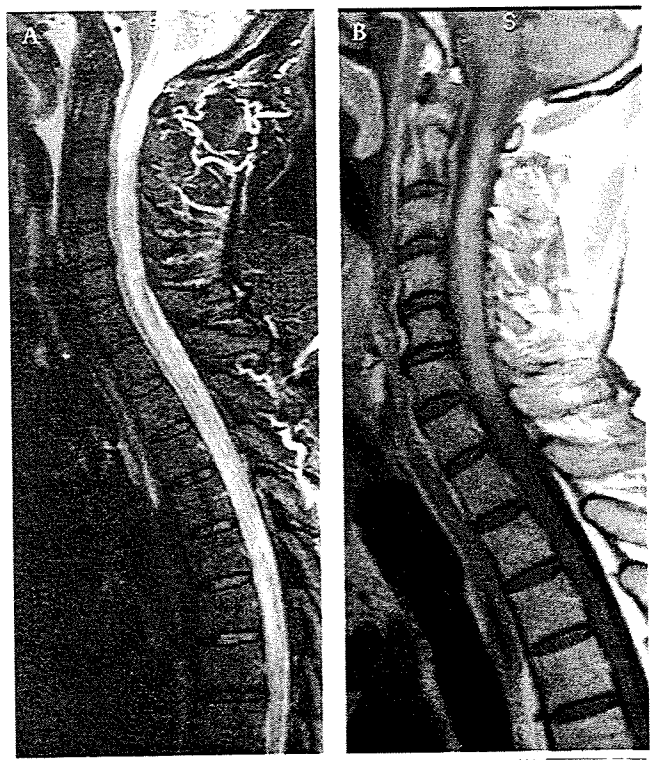

Figure 1 Sixty-five-year-old male patient, who presented with hiccough and rapidly developed complete paraplegia, sphincter dysfunction and respiratory failure. He had three episodes of recurrent myelopathy and died of respiratory failure. Spinal MRI demonstrated a T2 hyperintense lesion extending from the medulla to approximately T9 (A) with gadolinium enhancement on the T1 image (B). Brain MRI was normal except for the contiguous medullary lesion. Antibody against aquaporin-4 (AQP4-IgG) was negative.

by Tartaglino *et al* in a study of 68 MS cases in the USA.[7] These figures contrast markedly with a frequency up to 30% in Asian MS cases.[6] This may reflect the higher incidence of optic-spinal forms of MS in Asia.[9] The overlapping of clinical findings in the present study lends support to the concept of a single disorder with a heterogeneous spectrum of manifestations.[6 9–14]

Our findings indicate that LEM may be the initial presentation of NMO or CMS, or may develop later in patients with clinically isolated syndromes. Based on our findings, when LEM is the first manifestation, a number of MRI features may be at least partially helpful in predicting whether the clinical course will be that of CMS or NMO. First, longer lesions are more predictive of NMO, particularly when located in the thoracic cord and associated with cord swelling and contrast enhancement, whereas shorter lesions in the cervical cord are more predictive of CMS. Unlike Matsuoka *et al*[8] we did not find the axial location and extent of the cord lesion helpful in distinguishing cases of NMO and CMS. Second, the presence of cerebral lesions at the initial presentation is suggestive of CMS, and it is noteworthy that none of our patients with LEM onset and normal brain MRI went on to develop CMS over the period of follow-up of 3 to 11 years. In contrast with the typical brain lesions in cases of CMS, the cerebral lesions in NMO and isolated LEM may be unusual, such as tumefactive and medullary lesions.

Contrary to expectations, the prognosis of patients with LEM was found to be variable. Although in NMO cases LEM was usually associated with a severe disability and poor outcome, mild or moderate disability was observed in 42% of patients in the group as a whole, and 8% of cases had a benign course even after intervals of over 10 years. A benign prognosis has also been reported in children with LEM.[15] The present findings therefore indicate that LEM is not always associated with a poor outcome, particularly in patients with CMS.

It has been suggested that monophasic or recurrent isolated LEM, as well as recurrent isolated optic neuritis, should be included in a broader NMO spectrum based on the presence of AQP4-IgG,[16] and the term "NMO spectrum" has frequently been used. In our cohort, AQP4-IgG testing was not as helpful as expected, and Marignier *et al* found similarly that in their cohort AQP4-IgG positivity did not correlate with any clinical or radiological features in their NMO cases.[10] Only one of 20 of our cases tested had AQP4-IgG (0 of 4 with NMO), but these results are entirely consistent with other reports from Canada, India and Singapore using equally sensitive assays.[17–19] The low seropositivity of AQP4-IgG in our study may be due to immunogenetic differences, as the 20 patients tested for AQP4-IgG were exclusively Caucasian. In contrast, 26% of LEM in a Japanese cohort were seropositive assayed in the same laboratory.[8] Moreover, the Mayo Clinic cohort was about 50% non-Caucasian with variable amounts of African and American Indian origin patients.[16] Although six of the 26 LEM patients were not tested for AQP4-IgG, this did not affect our overall results, which are of significance for further investigation of AQP4-IgG in LEM in other Caucasian cohorts, as it is evident that there remain numerous unexplained discrepancies in the published AQP4-IgG data.[14]

In summary, LEM did occur in CMS, as well as NMO, could be the initial presentation of either condition and could also occur in isolation. Patients with LEM had highly heterogeneous clinical characteristics and a low incidence of AQP4-IgG seropositivity in this population.

Acknowledgements F Christiansen, A Castley, S Pummer and the staff at the Department of Clinical Immunology & Immunogenetics Royal Perth Hospital, PathWest kindly assisted with sample processing and data collection.

Funding W Qiu was supported by Endeavour International Postgraduate Research Scholarships (EIPRS) of Australia and Postgraduate Award from University of Western Australia. J-S Wu was supported by Medical Postgraduate Scholarship from Multiple Sclerosis Research Australia and the National Health & Medical Research Council of Australia.

Competing interests None.

Ethics approval Ethics approval was provided by University of Western Australia.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Wingerchuk DM,** Lennon VA, Pittock SJ, *et al.* Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006;**66**:1485–9.
2. **McDonald WI,** Compston A, Edan G, *et al.* Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;**50**:121–7.
3. **Wingerchuk DM,** Hogancamp WF, O'Brien PC, *et al.* The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology* 1999;**53**:1107–14.
4. **Bot JC,** Barkhof F, Polman CH. Spinal cord abnormalities in recently diagnosed MS patients: added value of spinal MRI examination. *Neurology* 2004;**62**:226–33.
5. **Kira J,** Kanai T, Nishimura Y, *et al.* Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. *Ann Neurol* 1996;**40**:569–74.
6. **Chong H,** Ramli N, Lee K. Magnetic resonance imaging of Asians with multiple sclerosis was similar to that of the West. *Can J Neurol Sci* 2006;**33**:95–100.
7. **Tartaglino LM,** Friedman DP, Flanders AE, *et al.* Multiple sclerosis in the spinal cord: MR appearance and correlation with clinical parameters. *Radiology* 1995;**195**:725–32.
8. **Matsuoka T,** Matsushita T, Kawano Y, *et al.* Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. *Brain* 2007;**130**:1206–23.

9. **Matsuoka T,** Matsushita T, Osoegawa M, *et al.* Heterogeneity and continuum of multiple sclerosis in Japanese according to magnetic resonance imaging findings. *J Neurol Sci* 2008;**266**:115–25.
10. **Marignier R,** de Seze J, Vukusic S, *et al.* NMO-IgG and Devic's neuromyelitis optica: A French experience. *Mult Scler* 2008;**14**:440–5.
11. **Nakashima I,** Fujihara K, Miyazawa I, *et al.* Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. *J Neurol Neurosurg Psychiatry* 2006;**77**:1073–5.
12. **Giovannoni G.** To test or not to test? NMO-IgG and optic neuritis. *Neurology* 2008;**70**:2192–3.
13. **Wu JS,** Zhang MN, Carroll WM, *et al.* Characterisation of the spectrum of demyelinating disease in Western Australia. *J Neurol Neurosurg Psychiatry* 2008;**79**:1022–6.
14. **Kermode AG.** Aquaporin-4 IgG: Overview and future perspectives. *Neurol Asia* 2008;**13**:179–83.
15. **Andronikou S,** Albuquerque-Jonathan G, Wilmshurst J, *et al.* MRI findings in acute idiopathic transverse myelopathy in children. *Pediatr Radiol* 2003;**33**:624–9.
16. **Weinshenker BG,** Wingerchuk DM, Vukusic S, *et al.* Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. *Ann Neurol* 2006;**59**:566–9.
17. **Kevin T,** Shuguna M, Kong Y, *et al.* The seroprevalence of NMO-IgG in Singapore: A pilot study. *Neurol Asia* 2008;**13**:205–6.
18. **Pandit L.** Neuromyelitis optica antibody (NMO IgG) status in Indian patients with multiple sclerosis and allied demyelinating disorders. *Neurol Asia* 2008;**13**:175–8.
19. **Horga A,** Hemmer B, Edan G. antibodies to aquaporin-4 in patients with a clinically isolated syndrome: Analysis from the BENEFIT study. *Mult Scler* 2008;**14**:S29.

SHORT REPORT *Multiple Sclerosis* 2009; **15**: 1113–1117

Extensive vasogenic edema of anti-aquaporin-4 antibody-related brain lesions

T Matsushita[1], N Isobe[1], T Matsuoka[1], T Ishizu[1], Y Kawano[1], T Yoshiura[2], Y Ohyagi[1] and J Kira[1]

Objective Using neuroimaging, we analyzed the nature of extensive brain lesions in five anti-aquaporin-4 (AQP4) antibody-positive patients with neuromyelitis optica spectrum disorders.
Results Extensive brain lesions involved white matter in three, and basal ganglia and corpus callosum in one each. Four patients showed high diffusivity on apparent diffusion coefficient maps and three demonstrated increased choline/creatine ratios and decreased *N*-acetyl-aspartate/creatine ratios on ^{1}H-magnetic resonance spectroscopy. These findings suggested that the lesions were vasogenic edema associated with inflammation. Unusual brain symptoms associated with such lesions included recurrent limbic encephalitis, parkinsonism, and coma.
Conclusion Anti-AQP4 antibody is considered to be associated with the neuroimaging appearances of vasogenic edema. *Multiple Sclerosis* 2009; **15**: 1113–1117. http://msj.sagepub.com

Key words: aquaporin-4; diffusion-weighted image; MRI; MRS; neuromyelitis optica; vasogenic edema

Introduction

Neuromyelitis optica (NMO) is characterized by severe and selective involvement of the optic nerves and spinal cord [1]. However, a specific IgG against NMO, designated NMO-IgG [2], recognizing aquaporin-4 (AQP4) [3], has changed the concept of NMO. Some patients with NMO-IgG show atypical brain lesions, such as large confluent and diencephalic lesions [4]. In fact, 60% of patients fulfilling the 1999 criteria for NMO show brain lesions on MRI [4]. Thus, the 2006 revised criteria for NMO include the presence of NMO-IgG and do not preclude patients with brain lesions [5]. However, the nature of anti-AQP4 antibody-related brain lesions is ill-defined. We report five cases of NMO spectrum disorders with extensive brain lesions characterized by apparent diffusion coefficient (ADC) maps and magnetic resonance spectroscopy (MRS).

Methods

Patients

Demographic features of five cases are given in Table 1.

Case 1

A 62-year-old female with a 40-year history of recurrent optic neuritis and myelitis suddenly developed agitation, abnormal behavior, and a confused state. Her anti-AQP4 antibody titer was 1:16,384 [6]. She had previously shown no brain symptoms, despite the presence of hazy periventricular white matter lesions (Figure 1A,B). She now showed bilateral extensive white matter lesions that were far more severe than those revealed on previous MRI scans,

[1]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Division of Neuroradiology, Department of Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
Correspondence to: Jun-Ichi Kira, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashiku, Fukuoka 812-8582, Japan.
Email: kira@neuro.med.kyushu-u.ac.jp
Received 31 January 2009; accepted 8 April 2009

10.1177/1352458509106613

Table 1 MRI and MRS findings of extensive brain lesions in anti-AQP4 antibody-positive patients

Case No.	Age (years)/ sex	Anti-AQP4 antibody titer	Symptoms due to brain lesions	Optic neuritis	ATM	LESCL-s	Therapy	Effect of therapy	MRI				MRS		
									T2WI	T1WI	Contrast-enhancement	DWI	ADC map	Cho/Cr	NAA/Cr	Lactate
1	62/F	1:16,384	Delirium	+ (Bil.)	+	+	Steroid pulse + PE	+	Hyperintense	Isointense	—	Iso to hyperintense	Increased diffusivity	Increased	Decreased	—
2	25/M	1:8192	Depression bradykinesia, lethargy	+ (Lt.)	—	—	Steroid pulse + PE	+	Hyperintense	Isointense with patchy hyperintensiy	+	Isointense (partially hyperintense)	Increased diffusivity	Increased	Decreased	+
3	60/F	1:1024	Coma, seizure	+ (Bil.)	—	+	Steroid pulse	+	Hyperintense	Isointnse	—	Hyperintense	Increased diffusivity	NE	NE	NE
			Gait disturbance				Steroid pulse	+	Hyperintense	Isointnse	—	Slightly hyperintense	Increased diffusivity	NE	NE	NE
4	43 / F	1:1024	Lt. hemiparesis	+ (Rt.)	—	+	Steroid pulse + PE	+	Hyperintense	Hypointense	—	Hypointense encircled with hyperintensity	Increased diffusivity	Increased	Decreased	+
5	51/F	1:4096	Headache, nausea	+ (Bil.)	—	+	Steroid pulse	+	Hyperintense	Isointense	—	NE	NE	NE	NE	

Case 3 twice showed extensive brain lesions. MRI findings for case 4, including DWI, ADC map and MRS results, were previously reported as supplementary figures [6]. Bil., bilateral; Lt., left; NE, not examined; PE, plasma exchange; Rt., right; T1WI, T1-weighted images; T2WI, T2-weighted images.

especially in both temporal lobes (Figure 1C,D). These lesions demonstrated hyperintensity on T2-weighted, isointensity on T1-weighted and hyperintensity on fluid-attenuated inversion recovery (FLAIR) images, but no contrast enhancement. The extensive white matter lesions showed increased diffusivity on the ADC map (Figure 1F), being hypointense on diffusion-weighted images (DWIs) (Figure 1G). Although corticosteroid pulse therapy had no effect, three courses of plasma exchange rapidly improved her symptoms and the white matter lesions, which mostly resolved to prerelapse levels (Figure 1H) leaving no brain symptoms. After finishing therapy, the anti-AQP4 antibody titer fell to 1:2048. However, 3 months later, she again developed agitation, abnormal behavior, and a confused state. Although limbic encephalitis symptoms were as severe as the previous time, temporal lobe lesions were not enlarged and anti-AQP4 antibody was not elevated (1:256). Three courses of plasma exchange had no beneficial effect, while intravenous high-dose immunoglobulin administration (15 g/day for five consecutive days) alleviated her limbic encephalitis symptoms 2 months after initiation of her second episode of limbic encephalitis.

Case 2

A 25-year-old man with Sjögren's syndrome, with four previous episodes of left optic neuritis and three of paresthesia in all four limbs for the preceding 3 years, developed a high fever, butterfly rash, oral ulcers, and lymph node swelling. He had pancytopenia and anti-nuclear antibodies. His diagnosis was revised to systemic lupus erythematosus (SLE). Although his erythema and hematological disturbances were improved by the adminstration of high-dose oral corticosteroid, he developed bradykinesia, weakness of both lower limbs, urinary incontinence and then a disturbance of consciousness. Neurologically, he was lethargic and had cogwheel rigidity in the neck and four limbs, bradykinesia, mild quadriparesis with mild hyperreflexia and extensor plantar responses on both sides. His anti-AQP4 antibody titer was 1:8192. Scattered high-signal intensity lesions in the bilateral basal ganglia and cerebral white matter were seen on MRI (Figure 1K–N). High-signal dots scattered in bilateral basal ganglia on T1-weighted images probably reflected exudates from blood vessels. By contrast, the one in the left caudate head appeared hemorrhagic because the lesion was hypointense on T2* images (Figure 1O). Additional contrast-enhanced spots were also visible. Bilateral basal ganglia showed increased diffusivity on ADC (Figure 1P) and were isointense on DWIs (Figure 1Q). His symptoms and MRI lesions did not respond to

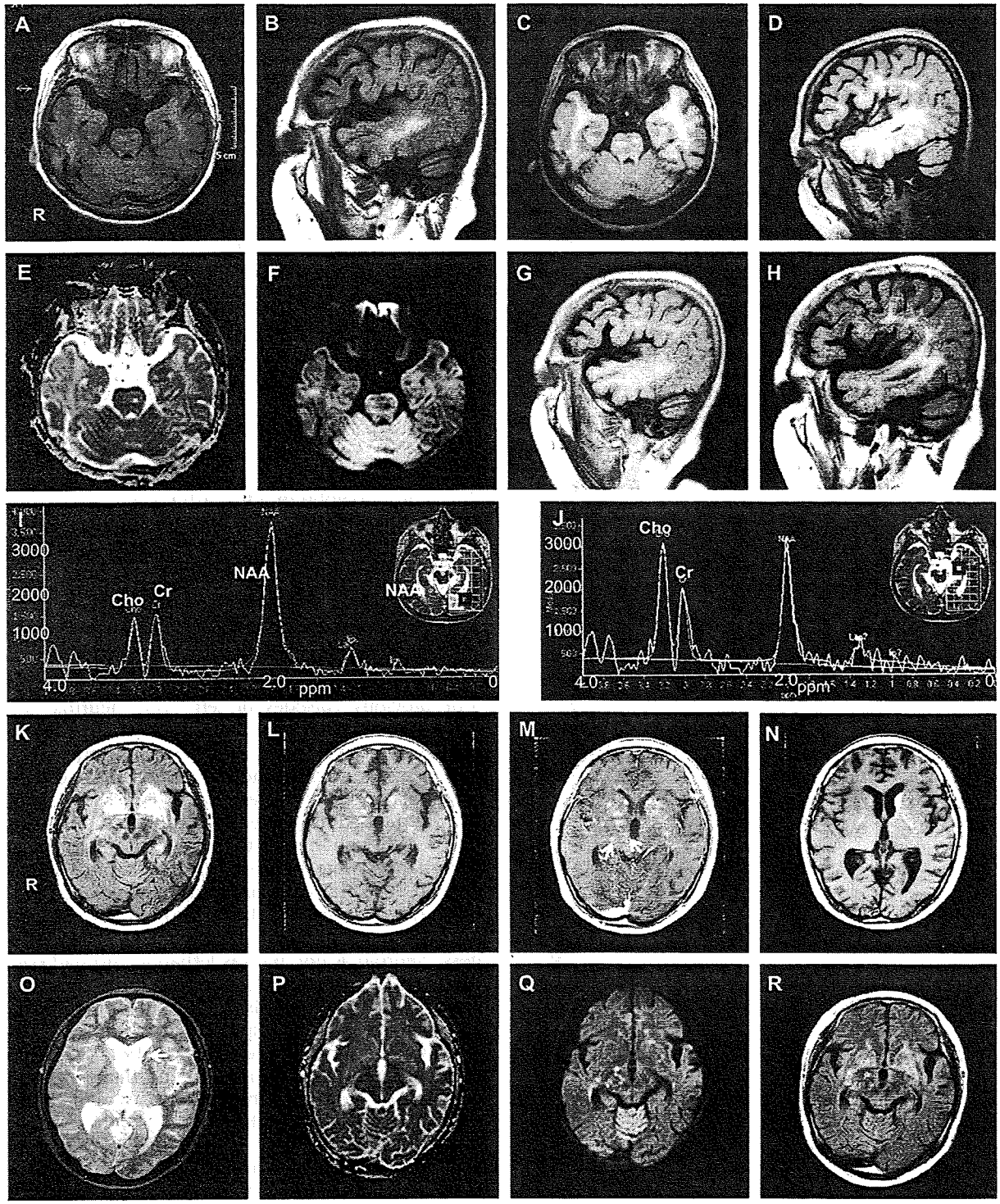

Figure 1 The extensive brain lesions in case 1 seen on MRI and MRS (A–J). (A,B) FLAIR image, showing mild hyperintensity in both temporal lobes, taken 5 months prior to relapse. (C,D) The temporal lobe lesions become extensive at relapse. (E) ADC map showing high diffusivity in both temporal lobes. (F) DWI showing hypointensity in the temporal lobes. (G) Although two courses of methylprednisolone pulse therapy (1 g/day for 3 days) followed by intravenous administration of hydrocortisone (50 mg/day) had no effects, the patient promptly improved following three courses of plasma exchange

44 days after the onset of relapse; the lesions returned to the prerelapse level on FLAIR images. (H) No apparent new hyperintensity lesion is seen on a FLAIR image taken at the recurrence of limbic encephalitis symptoms. (I) On MRS, the Cho/Cr ratio of normal-appearing white matter is 0.54 and the NAA/Cr ratio is 5.18. (J) Left temporal lobe lesions show a high Cho/Cr (1.70) and low NAA/Cr (1.90) ratios compared with normal-appearing white matter. The voxel on MRS is shown as a square box in the inset. The extensive brain lesions in case 2, as in Table 1, seen on MRI (K–Q). (K) Bilateral basal ganglia show hyperintensity on a FLAIR image. (L) High-signal intensity dots are scattered in bilateral basal ganglia, probably reflecting exudates from blood vessels, on a T1-weighted image. (M) On a gadolinium-enhanced T1-weighted image, additional contrast-enhanced spots (arrowheads) are visible. (N) The left caudate head shows hyperintensity on a T1-weighted image. (O) On a T2* image (arrowhead) taken 2 months after the onset of parkinsonism, the left caudate head shows hypointensity, suggesting hemorrhage. (P) ADC map showing high diffusivity in the bilateral basal ganglia. (Q) DWI showing isointensity in the basal ganglia. (R) Hyperintensity in the bilateral basal ganglia on FLAIR images improves after plasma exchange. ADC, apparent diffusion coefficient; Cho, choline; Cr, creatine; DWI, diffusion-weighted images; FLAIR, fluid-attenuated inversion recovery images; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; R, right.

methylprednisolone pulse therapy, but did rto three courses of plasma exchange with amelioration of brain MRI lesions 46 days after the start of parkinsonism (Figure 1R).

Neuroimaging

All MRI studies were performed using 1.5-T Magnetom Vision and Symphony units (Siemens Medical Systems, Erlangen, Germany) as described previously [6]. Using a 3-Tesla superconducting MR unit (Achieva 3.0T; Philips, Eindhoven, Netherlands) with a standard head coil, water-suppressed ^{1}H-MRS was obtained after global and voxel shimming using chemical shift imaging (multivoxel MR spectroscopic imaging) based on the spin-echo technique, as described previously [7].

Results

The six extensive brain lesions (>3 cm) seen in the five patients were all T2-high and T1-iso-to-low (Table 1). Among these, the five lesions examined had increased diffusivity on ADC and three of them showed hypo- or isointensity on DWIs while the other two were hyperintense, probably the result of T2 shine-through. Contrast-enhancement was absent or faint in five and dotted in one. MRS showed increased Cho/Cr and decreased NAA/Cr ratios in all three patients examined (Figure 1I,J). A lactate peak was also observed in two.

Discussion

All the extensive brain lesions in our patients with anti-AQP4 antibody demonstrated increased diffusivity on ADC, suggesting the occurrence of vasogenic edema. On MRS, the increased Cho/Cr ratio and the decreased NAA/Cr ratio, together with the emergence of a lactate peak in some, were compatible with acute inflammation [8]. Therefore, it seems reasonable to assume that extensive brain lesions in patients with anti-AQP4 antibody largely consist of profuse vasogenic edema associated with acute inflammation. In AQP4 knock-out mice, cytotoxic edema is ameliorated [9] while vasogenic edema worsens [10]. Destruction of AQP4 on astrocyte foot processes by complement-activation by anti-AQP4 antibody may retard the resolution of vasogenic edema.

In case 1, the recurrence of limbic encephalitis came without any increase of anti-AQP4 antibody titers. As AQP4 constitutes part of the blood brain barrier (BBB), once an astrocyte foot process is destroyed by anti-AQP4 antibody, the BBB may become leaky. Therefore, subsequent relapses may be easily induced despite low titers of anti-AQP4 antibody. Alternatively, because of the absence of high titers of anti-AQP4 antibody, it is possible that the second attack of limbic encephalitis was the result of acute inflammation without severe vasogenic edema.

In case 2, the basal ganglia were extensively involved, unlike in NMO spectrum disorders, and the case was assumed to have cerebral vasculitis associated with SLE. The anti-AQP4 antibody may have been produced secondary to the vasculitis.

Acknowledgements

This work was supported in part by grants from the Research Committees of Neuroimmunological Diseases, the Ministry of Health, Labour and Welfare, Japan and from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
Disclosure: The authors report no conflicts of interest.

References

1. de Seze, J. Neuromyelitis optica. *Arch Neurol* 2003; **60**: 1336–1338.
2. Lennon, VA, Wingerchuk, DM, Kryzer, TJ, *et al.* A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; **364**: 2106–2112.
3. Lennon, VA, Kryzer, TJ, Pittock, SJ, Verkman, AS, Hinson, SR. IgG marker of optic-spinal multiple sclerosis

binds to the aquaporin-4 water channel. *J Exp Med* 2005; **202**: 473–477.
4. Pittock, SJ, Lennon, VA, Krecke, K, Wingerchuk, DM, Lucchinetti, CF, Weinshenker, BG. Brain MRI abnormalities in neuromyelitis optica. *Arch Neurol* 2006; **63**: 390–396.
5. Wingerchuk, DM, Lennon, VA, Pittock, SJ, Lucchinetti, CF, Weinshenker, BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; **66**: 1485–1489.
6. Matsuoka, T, Matsushita, T, Kawano, Y, *et al.* Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. *Brain* 2007; **130**: 1206–1223.
7. Mihara, F, Kuwabara, Y, Yoshida, T, *et al.* Correlation between proton magnetic resonance spectroscopic lactate measurements and vascular reactivity in chronic occlusive cerebrovascular disease: a comparison with positron emission tomography. *Magn Reson Med* 2000; **18**: 1167–1174.
8. Simone, IL, Tortorella, C, Federico, F, *et al.* Axonal damage in multiple sclerosis plaques: a combined magnetic resonance imaging and ^{1}H-magnetic resonance spectroscopy study. *J Neurol Sci* 2001; **182**: 143–150.
9. Manley, GT, Fujimura, M, Ma, T, *et al.* Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat Med* 2000; **6**: 159–163.
10. Papadopoulos, MC, Manley, GT, Krishna, S, Verkman, AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J* 2004; **18**: 1291–1293.

Proposed modifications to the McDonald criteria for use in Asia

In the latest revision to McDonald diagnostic criteria for multiple sclerosis (MS), the authors commented that the Asian neurological community will determine whether the Criteria can be generalized to their population and how modifications to the Criteria will make them more appropriate in the Asian population [1]. In 2006, a group of leading Asian neurologists with an interest in MS proposed some modifications to the McDonald criteria for Asians with MS (see Table 1) [2]. The proposal was based on MS as an idiopathic inflammatory demyelinating disease disseminated in time and place.

For the spinal magnetic resonance imaging (MRI), instead of stating that the lesion should be under two vertebral segments in length, our proposal was to place no restriction on the length of the spinal cord lesion [1–3]. This is because long spinal cord lesions are frequently seen in Asian with classical as well as optic-spinal MS. For example, in a retrospective joint Asian MRI study of MS patients who fulfilled Poser criteria, it was found that in 86 patients, spinal cord lesions had a mean length of 3.5 + 3.3 vertebral segments. Forty-seven percent of the cord lesions were longer than two vertebral segments in length, 29% in patients with classical MS, and 52% in those with optic-spinal MS [4]. For spinal cord swelling, the McDonald criteria stated that there should be no swelling of the cord and that the lesion should occupy only part of the cross section of the cord. In the same joint Asian MRI study, cord swelling was seen in 23% of the lesions, 13% in classical MS, and 29% in optic-spinal MS; complete cross sectional involvement was also seen in 23% of the lesions, 7% in classical MS, and 33% in optic-spinal MS [4,5]. We thus propose that spinal cord swelling and complete cross-sectional cord involvement should not exclude the diagnosis of MS in Asians.

The McDonald criteria require nine T2-hyperintense MRI brain lesions or one gadolinium-enhancing lesion as one of the criteria of dissemination in space. In the revised McDonald criteria, an enhancing spinal cord lesion is considered equivalent to an enhancing brain lesion [1,3]. In the joint Asian study, the McDonald criteria and its revised version had a sensitivity of only 49% and 52% among 66 Asians with MS [1,3,5]. The low sensitivity is partly due to fewer brain lesions in Asians with MS [4]. To improve the sensitivity of brain MRI in diagnosing MS in Asians, the proposed modification to the McDonald criteria was to accept four or more T2-hyperintense lesions in patients under the age of 60 years as being sufficient. The proposal was based on the joint Asian MRI study, where 69% of 101 patients had four or more T2-hyperintense brain lesions, 84% in 51 classical MS, and 52% in 50 optic-spinal MS [4]. Swanton, *et al.* [6] recently proposed more than one lesion in two or more typical sites (juxtacortical, periventricular, posterior fossa, and spinal cord) as criteria for dissemination in space. As 72% of the patients in the joint Asian MRI study would fulfill the Swanton Criteria (unpublished data), it would also be an acceptable alternative.

The McDonald criteria [3] require that the lymphocyte pleocytosis in cerebrospinal fluid (CSF) should be less than 50/mm^3. There have been a number of studies reporting that a significant proportion of Asian MS patients have a CSF lymphocytic pleocytosis of more than 50/mm^3 [7]. Although CSF pleocytosis of more than 50/mm^3 might suggest other diagnoses by itself, this should not unequivocally exclude the diagnosis of MS in

Table 1 Proposed modifications to McDonald diagnostic criteria for Asians with multiple sclerosis

McDonald diagnostic criteria [3]	Proposed modifications for Asians
Spinal MRI	
Spinal cord lesion should be under two vertebral bodies in height	No restriction to length of spinal cord lesion
No swelling of the spinal cord lesion	No restriction to spinal cord lesion with swelling
Spinal cord lesion should occupy part of the cross section	No restriction to spinal cord lesion involving complete cross section
Brain MRI	
Nine T2-hyperintense lesions or one gadolinium-enhancing lesion	Four or more brain MRI T2-hyperintense lesions in patients less than 60 years of age or one gadolinium-enhancing lesion; or one lesion in two or more typical sites (juxtacortical, periventricular, posterior fossa, and spinal cord)
Cerebrospinal fluid	
Lymphocyte pleocytosis should be <50/mm^3	No restriction on CSF pleocytosis
Disease restricted to spinal cord	
Dissemination of space should not be restricted to spinal cord	No restriction to lesions clearly separated in space but limited to spinal cord

MRI, magnetic resonance imaging; CSF, cerebrospinal fluid.

10.1177/1352458509104587

Asian patients. The McDonald criteria stated that dissemination of space should not be restricted to the spinal cord [3]. Relapses limited to the spinal cord account for a significant proportion of MS patients in Asia. A Malaysian report found 20% of patients had relapses limited to the spinal cord [8]. Therefore, Asian patients with two or more spinal cord lesions clearly separated in time and space was proposed to also be accepted as consistent with MS.

When these proposals were presented to a group of about 72 neurologists from the Asia Pacific region meeting in Taipei (Multiple Sclerosis Forum on November 2–4, 2007), 70% of them thought that the revised McDonald's criteria [1] needed to be modified if it was to be used in Asia. Seventy-one percent of the participants thought that either four or more T2-hyperintense lesions or Swanton's criteria [6] was sufficient to show dissemination in space. Two thirds of the participants agreed that there should be no restriction on the length of spinal cord lesions and 82% agreed that cord lesions with swelling or lesions that involved the entire cross-sectional area should still be considered consistent with MS. Fifty-five percent of the participants also agreed that CSF pleocytosis was not incompatible with the diagnosis of MS. Finally, 84% of the participants agreed that spinal cord lesions clearly separated on MRI should be accepted as evidence of dissemination in space in the diagnosis of MS. However, these proposals were based on retrospective studies as there is a lack of prospective studies. Prospective studies on the predictive values of various diagnostic criteria are sorely needed in this region.

As for neuromyelitis optica (NMO), the recently proposed diagnostic criteria are very similar to optic-spinal MS among Asians. As "whether NMO/optic-spinal MS should be regarded as a disease distinct from MS" is still uncertain [9], it is better to address this when more data are available.

References

1. Polman, CH, Reingold, SC, Edan, G, *et al.* Diagnostic criteria for multiple sclerosis: 2005 revision to the "McDonald Criteria". *Ann Neurol* 2005; **58**: 840–846.
2. Chong, HT, Li, PCK, Singhal, BS, *et al.* Proposed modifications to McDonald diagnostic criteria for Asians with multiple sclerosis. *Neurology Asia* 2006; **11**: 87–90.
3. McDonald, WI, Compston, A, Edan, G, *et al.* Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. *Ann Neurol* 2001; **50**: 121–127.
4. Chong, HT, Ramli, N, Lee, KH, *et al.* Magnetic resonance imaging of Asians with multiple sclerosis was similar to that of the West. *Neurology Asia* 2004; **9**: 47–53.
5. Chong, HT, Ramli, N, Lee, KH, *et al.* Low sensitivity of McDonald criteria in the diagnosis of multiple sclerosis among Asians. *Neurology Asia* 2006; **11**: 129–133.
6. Swanton, JK, Rovira, A, Tintore, M, *et al.* MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndrome: a multiple centre retrospective study. *Lancet Neurol* 2007; **6**: 677–686.
7. Kuroiwa, Y, Igata, A, Itahara, K, *et al.* National survey of multiple sclerosis in Japan – clinical analysis of 1,084 cases. *Neurology* 1975; **25**: 845–851.
8. Tan, CT. Multiple sclerosis in Malaysia. *Neurol J Southeast Asia* 1997; **2**: 1–5.
9. Kermode, A. Aquaporin-4 IgG: overview and future perspective. *Neurology Asia* 2008; **13**: 179–183.

HT Chong[1], J Kira[2], CP Tsai[3], B Ong[4], PCK Li[5], A Kermode[6] and CT Tan[1]

[1]*Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia*

[2]*Neurological Institute, Kyushu University, Fukuoka, Japan*

[3]*The Neurological Institute, Taipei Veteran General Hospital, Taipei, Taiwan*

[4]*Department of Medicine, National University of Singapore, Singapore*

[5]*Department of Medicine, Queens Elizabeth Hospital, Hong Kong, China*

[6]*Australia Neuromuscular Research Institute, Charles Gairdner Hospital, Nedlands, Australia*

Correspondence to: HT Chong, Neurology Laboratories, University of Malaya Medical Centre, Jalan Universiti, Lembah Pantai, 59100 Kuala Lumpur, Malaysia. Email: chongvictor@gmail.com

Journal of the Neurological Sciences 281 (2009) 34–40

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Multimodality-evoked potential study of anti-aquaporin-4 antibody-positive and -negative multiple sclerosis patients

Akihiro Watanabe [a,1], Takuya Matsushita [a,1], Hikaru Doi [a], Takashi Matsuoka [a], Hiroshi Shigeto [a,b], Noriko Isobe [a], Yuji Kawano [a], Shozo Tobimatsu [b], Jun-ichi Kira [a,*]

[a] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Japan
[b] Department of Clinical Neurophysiology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Japan

ARTICLE INFO

Article history:
Received 22 July 2008
Received in revised form 7 January 2009
Accepted 25 February 2009
Available online 1 April 2009

Keywords:
Anti-aquaporin-4 antibody
Multiple sclerosis
Neuromyelitis optica
Opticospinal form
Visual-evoked potential
Sensory evoked potential
Motor evoked potential

ABSTRACT

Neuromyelitis optica (NMO) is claimed to be a distinct disease entity from multiple sclerosis (MS) because of its strong association with NMO-IgG/anti-AQP4 antibody; however, the in vivo role of the antibody remains unknown. Therefore, we aimed to clarify whether the presence of anti-AQP4 antibody is associated with any abnormalities in multimodality-evoked potentials in 111 patients with relapsing–remitting or relapsing-progressive MS, including the opticospinal form of MS, 18 of whom were seropositive for anti-AQP4 antibody. More patients with anti-AQP4 antibody showed a lack of the P100 component on visual-evoked potentials (VEPs) 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%, $p=0.021$). The frequencies of non-responses and delayed central sensory conduction times in median and posterior tibial nerve somatosensory-evoked potentials (SEPs) were not significantly different between anti-AQP4 antibody-positive and -negative patients. In terms of upper and lower limb motor-evoked potentials (MEPs), the frequencies of non-responses and delayed central motor conduction times did not differ significantly based on the presence or absence of anti-AQP4 antibody. The frequency of optic nerve lesions on MRI was significantly higher in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients ($p=0.0137$). Multiple logistic analyses revealed that anti-AQP4 antibody positivity (OR = 8.406, $p=0.02$) and unevoked VEP responses (OR = 35.432, $p<0.001$) were significantly related to development of severe visual impairment. Such an association of anti-AQP4 antibody with disability was not found for either severe motor or sensory impairment. These findings suggest a distinctive nature of optic nerve lesions between anti-AQP4 antibody-positive and -negative patients; lesions are supposed to be more necrotic in the former group and more demyelinating in the latter.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that is generally considered to be mediated by myelin-autoreactive T cells. In Asians, selective and severe involvement of the optic nerves and spinal cord is characteristic [1]. There are two distinct subtypes of MS: the opticospinal form (OSMS), which has similar features to the relapsing–remitting form of neuromyelitis optica (NMO) in Western populations [2–5], and the conventional form (CMS), which is similar to classical MS in Western populations [1,2,6,7].

Recently, a specific IgG expressed by NMO patients, designated NMO-IgG, was described [8]; its relevant antigen was reported to be aquaporin-4 (AQP4) [9]. Because of the high specificity of NMO-IgG/anti-AQP4 antibody, NMO has been claimed to be a distinct disease entity with a fundamentally different causal mechanism from MS [10]. In a selected series of Japanese patients with OSMS, Nakashima et al. [11] reported an NMO-IgG positivity rate of approximately 60%, and we and others found that anti-AQP4 antibody is present in about 40 to 60% of OSMS patients [12]. Thus OSMS has now been suggested to be the same disease as NMO [13]. Additionally, selective loss of AQP4 from acute lesions in autopsied OSMS spinal cord specimens has been described [14,15]. Therefore, it is hypothesized that anti-AQP4 antibody is a causative agent for both NMO and OSMS and that demyelination is secondarily produced following damage to the astrocyte foot process, where AQP4 is localized [14–16].

Longitudinally extensive spinal cord lesions (LESCLs) extending over three or more vertebral segments [3,10] are considered to be characteristic of NMO; however, in Asians, LESCLs are observed in one-

* Corresponding author. Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5340; fax: +81 92 642 5352.
E-mail address: kira@neuro.med.kyushu-u.ac.jp (J. Kira).
[1] These authors contributed equally to this work.

0022-510X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2009.02.371