Figure 6. Heterogeneity between perivascular deposition of activated complement and immunoglobulins and AQP4 expression. **(A–G)** Serial sections of the peripheral area of chronic active lesions in the cerebral white matter of NMO-10. **A.** Demyelinating lesions confined to the vicinity of blood vessels. **B.** No inflammatory cell infiltration. **C.** AQP4 immunoreactivity on astrocyte processes. Immunoreactivities to activated complement (C3d (**D**) and C9neo (**E**)) and immunoglobulins (IgM (**F**) and IgG (**G**)) in the perivascular areas. Note that one blood vessel does not show deposition of activated complement or immunoglobulins (arrow in **D–G**). **(H–L)** Serial sections in the cerebral white matter of the same case. **H.** A chronic inactive demyelinating lesion. **I.** Few CD68-positive macrophages in the lesion. **J.** AQP4 labeling of the astrogliosis covering the lesions. C3d (**K**) and IgG (**L**) immunoreactivities are most intense in the perivascular area. **(M–O)** Serial sections of a chronic active demyelinating lesion in the spinal cord of the same case. **M.** Loss of AQP4 immunoreactivity is confined to the perivascular areas associated with C3d (**N**) and IgM (**O**) deposition. **A, H,** KB, **B, I,** CD68 immunohistochemistry (IHC); **C, J, M,** AQP4 IHC; **D, K, N,** C3d IHC; **E,** C9neo IHC; **F, O,** IgM IHC; **G, L,** IgG IHC. Scale bar = 100 μm (**A–G**); 50 μm (**H–O**); 15 μm (**C**). AQP4 = aquaporin-4; GFAP = glial fibrillary acidic protein; KB = Klüver-Barrera staining; NMO = neuromyelitis optica.

complement-related mechanisms are important in AQP4 astrocytopathy in a fraction of NMO cases, as seen in one case with anti-AQP4 antibody, but AQP4 loss can also occur in some actively demyelinating MS lesions, and can occasionally be very extensive, as seen in the Baló-like concentric spinal cord lesions of MS-4. Furthermore, the lesion-to-lesion heterogeneity in the AQP4 expression pattern observed in NMO cases implies a heterogeneous relationship between anti-AQP4 antibody and loss of AQP4 expression.

Our study has some limitations inherent to studies using archival autopsied materials. First, because the autopsied cases died with the disease, there was a potential bias toward severe cases. Our histological evaluation focusing on early active lesions and separately analyzing necrotic lesions can minimize this selection bias. Second, the anti-AQP4 antibody status was only known in one case, because the other autopsies were performed before the discovery of NMO-IgG. These limitations are common to the other pathological studies on AQP4 expression in NMO by Misu *et al* (38) and Roemer *et al* (45). We were able to evaluate AQP4 expression in the one NMO case with confirmed anti-AQP4 antibodies, which enabled a detailed evaluation of the relationship between the presence of anti-AQP4 antibody and AQP4 expression in the CNS.

The results of our study are somewhat discordant with those of previous studies (38, 45), which might, in part, be attributable to the differences in methodology and materials. Regarding the staining method, in our previous (33) and present studies, we found AQP4 expression patterns similar to those reported in normal and diseased control brain tissues (1, 27, 38, 45) despite using a different anti-AQP4 antibody. The evaluation of necrotic lesions also requires some consideration. In necrotic lesions totally replaced by macrophages where no viable astrocytes existed, we could not differentiate whether the loss of AQP4 was caused by down-modulation of AQP4 in astrocytes or the loss of astrocytes *per se*. Furthermore, in such destructive lesions, it would be difficult to determine the causal relationship between AQP4 loss and the astrocyte damage. Therefore, we decided to put more stress on evaluating earlier lesions where the background tissues were still relatively preserved. Although the clinical features of MS are somewhat different between northern (Misu's cases) and southern Japanese (our cases) (15, 41), these differences in methodology should not seriously distort our results.

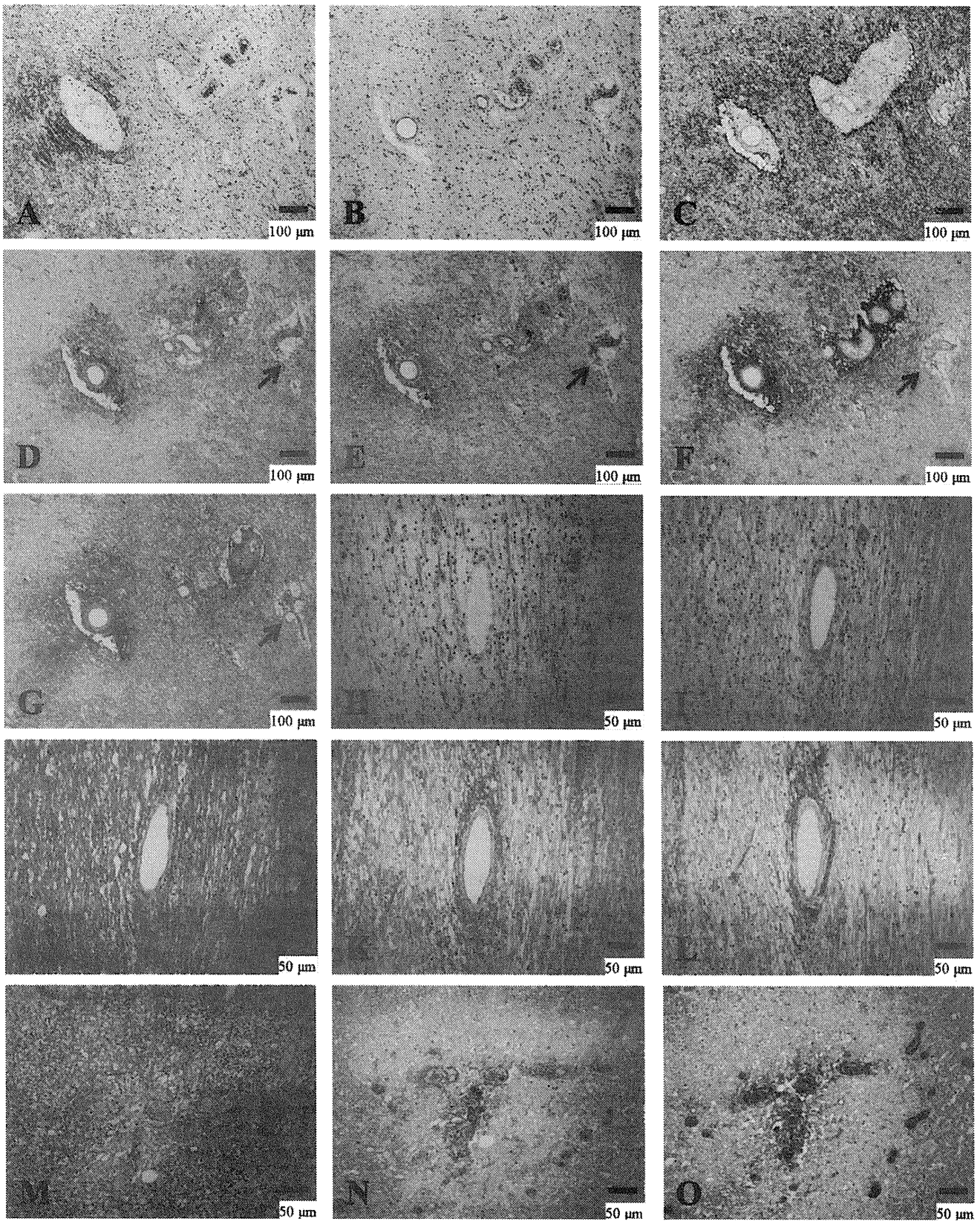
Half of our NMO cases showed a preferential loss of AQP4 in actively demyelinating and chronic active lesions, whereas the rest did not, despite severe tissue destruction. This is in line with the fact that anti-AQP4 antibody is not detected in 30%–70% of NMO cases (22). Vasculocentric complement and immunoglobulin deposition was noted only in NMO/NMOSD cases but not in MS cases. These findings support the notion that AQP4 loss in NMO cases results from immune responses to AQP4 (38, 45). Accordingly, it is possible that pathologically, there are two types of NMO, namely AQP4 autoimmunity-related and AQP4 autoimmunity-unrelated, and the latter may correspond clinically to seronegative NMO (31, 35).

The vasculocentric deposition of complement and immunoglobulins was only detected in NMO patients with AQP4 loss, including one with anti-AQP4 antibody. This supports the hypothesis that anti-AQP4 antibody destroys perivascular astrocyte foot processes via complement-dependent mechanisms. However, more than half of the AQP4-down-modulated NMO lesions showed no vasculocentric deposition of complement and immunoglobulins. This might be caused by very transient complement and immunoglobulin deposition. We staged NMO lesions with myelin-laden macrophages as active according to the lesion staging in MS; however, it is possible that complement/immunoglobulin deposition rapidly disappears even in the presence of myelin-laden macrophages once it triggers the astrocytic damage. Alternatively, the findings might be interpreted, such that the anti-AQP4 antibody and complement-mediated mechanism is unique to NMO, but does not always accompany every lesion. Further studies are necessary to clarify the time course of complement/immunoglobulin deposition during the formation of NMO lesions in an experimental model.

Our pathological study also argues against autoimmune AQP4 destruction as the sole NMO mechanism. First, the severity of tissue destruction was unrelated to tissue AQP4 loss or preservation. Second, AQP4 expression was heterogeneous even in the same individual. Although chronic inactive lesions may restore AQP4 expression with astrogliosis, one NMO case showed preservation of AQP4 in actively demyelinating optic chiasmal lesions, despite reduced AQP4 expression in other CNS lesions. Third, the perivascular deposition of complement and immunoglobulins did not closely correlate with perivascular AQP4 loss.

Anti-AQP4 antibody titer could change from patient to patient and between different disease stages in the same patient, which might have affected AQP4 expression levels in the pathological lesions. However, in this case, the existence of actively demyelinating lesions without AQP4 loss in NMO patients showing preferential AQP4 loss in other active lesions suggests that anti-AQP4 antibody does not always play a primary role in initiating inflammatory lesions. Other factor(s) may be responsible for triggering the pathological features, which are then modulated by anti-AQP4 antibodies. To clarify the real consequences of this autoantibody, a prospective neuropathological study based on anti-AQP4 antibody status is required.

© 2011 The Authors; Brain Pathology © 2011 International Society of Neuropathology



© 2011 The Authors; Brain Pathology © 2011 International Society of Neuropathology

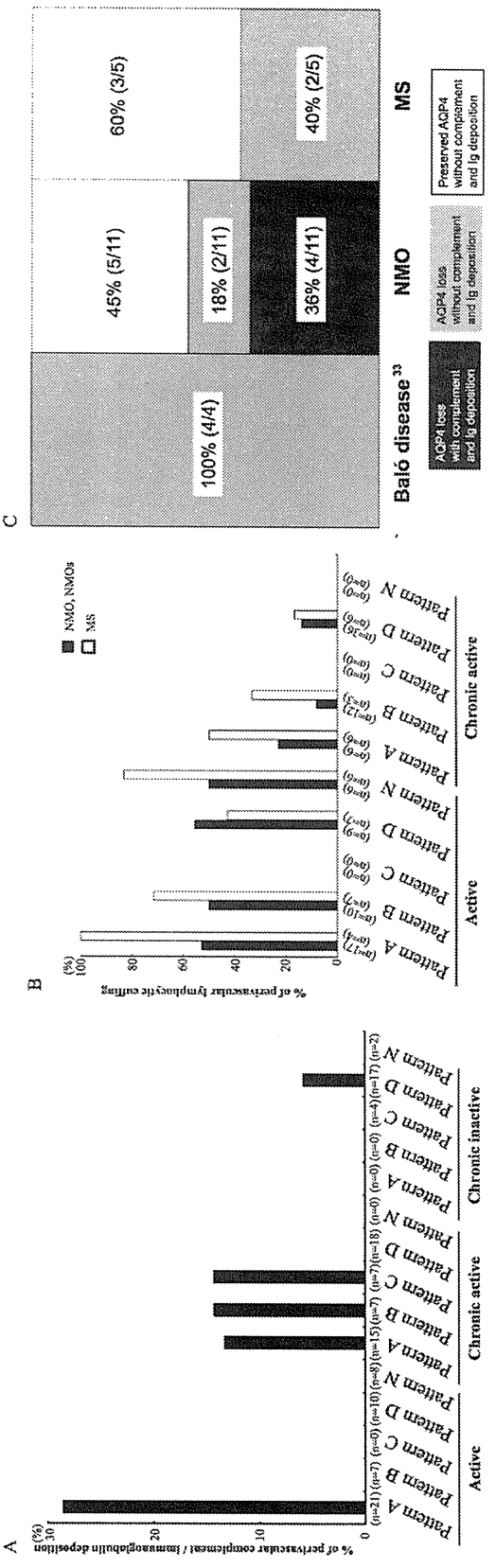


Figure 7. A. Positive rates of perivascular complement and immunoglobulin deposition in NMO/NMOSD cases according to AQP4 patterns and lesion stage. Because no MS case showed such depositions, only NMO/NMOSD lesions are included here. n = the number of demyelinating lesions. **B.** Positive rates of perivascular lymphocytic cuffing in the actively demyelinating and chronic active lesions according to AQP4 patterns and clinical phenotypes. n = the number of demyelinating lesions; see Table 3 for the definitions of the patterns. Any pattern with necrosis (Patterns X & N) was included in Pattern X. **C.** The relationship between AQP4 expression patterns and immunoglobulin/complement deposition in each disease type. The data on Baló's disease is cited from our previous report (33). AQP4 = aquaporin-4; MS = multiple sclerosis; NMO = neuromyelitis optica; NMOSD = NMO spectrum disorder.

© 2011 The Authors; Brain Pathology © 2011 International Society of Neuropathology

Regarding MS, somewhat inconsistent results have been reported. Misu *et al* (38) reported no loss of AQP4 in MS plaques, whereas Roemer *et al* (45) found stage-dependent loss of AQP4, with inactive MS lesions showing complete AQP4 loss. On the other hand, Sharma *et al* (48) observed patchy AQP4 loss only in a subset of active lesions following pattern III MS lesions. These authors found loss of perivascular astrocytic foot processes where AQP4 was lost, while numerous reactive astrocytes had intense AQP4 immunoreactivity in the lesions. In our study, AQP4 expression was decreased or totally lost in acute and chronic active demyelinating lesions in a fraction of MS cases, including one that lacked optic nerve and spinal cord lesions. At the cellular level, disruption of astrocytic vascular foot processes was evident in such lesions, and AQP4 expression was lost in both cell bodies and vascular foot processes of astrocytes as seen in acute NMO lesions. Our present and previous findings (33) raise the possibility that AQP4 loss *per se* is not confined to NMO, but rather, that it could also occur in actively demyelinating lesions of MS and BCS, suggesting a notion that astrocyte damage accompanied by AQP4 loss is not always related to anti-AQP4 antibody. Sharma *et al* (48) also reported that autoantibody-independent AQP4 loss and astrocytic dysfunction occurs in lipopolysaccharide-induced experimental demyelination, providing experimental support for such a notion. Interestingly, Baló-like concentric lesions in the spinal cord of MS-4 also showed similar extensive AQP4 loss to BCS, including in the areas of preserved myelin. We previously found no vasculocentric deposition of complement and immunoglobulin in BCS cases (33), and we and others (2) also demonstrated no such perivascular deposition in MS. Thus, extensive AQP4 loss without perivascular deposition of complement and immunoglobulin may be a common pathological feature of at least a subset of the concentric lesions in BCS and MS.

In summary, antibody-mediated AQP4 astrocytopathy occurs only in NMO, whereas antibody-independent AQP4 astrocytopathy can develop in various demyelinating conditions, including MS, BCS and a fraction of NMO cases (Figure 7C). Further studies on astrocytopathy as well as the dynamic plasticity of astrocytes in demyelinating diseases may shed light on the mechanisms underlying MS and allied disorders.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Research Committees of Neuroimmunological Diseases, the Ministry of Health, Labor and Welfare, Japan, and from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank Sachiko Nagae and Kimiko Sato, Department of Neuropathology, Kyushu University, for their excellent technical assistance, and Takekazu Ohi, Department of Neurology, Kurashiki Central Hospital, for providing materials.

REFERENCES

1. Aoki K, Uchihara T, Tsuchiya K, Nakamura A, Ikeda K, Wakayama Y (2003) Enhanced expression of aquaporin 4 in human brain with infarction. *Acta Neuropathol* **106**:121–124.
2. Barnett MH, Parratt JD, Cho ES, Prineas JW (2009) Immunoglobulins and complement in postmortem multiple sclerosis tissue. *Ann Neurol* **65**:32–46.
3. Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C *et al* (2009) Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol* **66**:617–629.
4. Bot JC, Barkhof F, Polman CH, Lycklama a Nijeholt GJ, de Groot V, Bergers E *et al* (2004) Spinal cord abnormalities in recently diagnosed MS patients: added value of spinal MRI examination. *Neurology* **62**:226–233.
5. Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M *et al* (2009) Neuromyelitis optica: pathogenicity of patient immunoglobulin *in vivo*. *Ann Neurol* **66**:630–643.
6. Cabrera-Gomez JA, Bonnan M, Gonzalez-Quevedo A, Saiz-Hinarejos A, Marignier R, Olindo S *et al* (2009) Neuromyelitis optica positive antibodies confer a worse course in relapsing-neuromyelitis optica in Cuba and French West Indies. *Mult Scler* **15**:828–833.
7. Chen CJ, Chu NS, Lu CS, Sung CY (1999) Serial magnetic resonance imaging in patients with Balo's concentric sclerosis: natural history of lesion development. *Ann Neurol* **46**:651–656.
8. Chong HT, Ramli N, Lee KH, Kim BJ, Ursekar M, Dayananda K *et al* (2006) Magnetic resonance imaging of Asians with multiple sclerosis was similar to that of the West. *Can J Neurol Sci* **33**:95–100.
9. Collongues N, Marignier R, Zephir H, Papeix C, Blanc F, Ritleng C *et al* (2010) Neuromyelitis optica in France: a multicenter study of 125 patients. *Neurology* **74**:736–742.
10. Fazio R, Malosio ML, Lampasona V, De Feo D, Privitera D, Marnetto F *et al* (2009) Antiacquaporin 4 antibodies detection by different techniques in neuromyelitis optica patients. *Mult Scler* **15**:1153–1163.
11. Graber JJ, Kister I, Geyer H, Khaund M, Herbert J (2009) Neuromyelitis optica and concentric rings of Balo in the brainstem. *Arch Neurol* **66**:274–275.
12. Hung TP, Landsborough D, Hsi MS (1976) Multiple sclerosis amongst Chinese in Taiwan. *J Neurol Sci* **27**:459–484.
13. Ikota H, Iwasaki A, Kawarai M, Nakazato Y (2010) Neuromyelitis optica with intraspinal expansion of Schwann cell remyelination. *Neuropathology* **30**:427–433.
14. Ikuta F, Koga M, Takeda S, Ohama E, Takeshita I, Ogawa H (1982) Comparison of MS pathology between 70 American and 75 Japanese autopsy cases. In: *Multiple Sclerosis East and West*, Y Kuroiwa, LT Kurland (eds), pp. 297–306. Kyushu University Press: Fukuoka.
15. Ishizu T, Kira J, Osoegawa M, Fukazawa T, Kikuchi S, Fujihara K *et al* (2009) Heterogeneity and continuum of multiple sclerosis phenotypes in Japanese according to the results of the fourth nationwide survey. *J Neurol Sci* **280**:22–28.
16. Itoyama Y, Tateishi J, Kuroiwa Y (1985) Atypical multiple sclerosis with concentric or lamellar demyelinated lesions: two Japanese patients studied post mortem. *Ann Neurol* **17**:481–487.
17. Jarius S, Franciotta D, Bergamaschi R, Wright H, Littleton E, Palace J *et al* (2007) NMO-IgG in the diagnosis of neuromyelitis optica. *Neurology* **68**:1076–1077.
18. Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM, Agre P (1994) Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc Natl Acad Sci U S A* **91**:13052–13056.
19. Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T *et al* (2009) Neuromyelitis optica: passive transfer to rats by human immunoglobulin. *Biochem Biophys Res Commun* **386**:623–627.
20. Kinoshita M, Nakatsuji Y, Moriya M, Okuno T, Kumanogoh A, Nakano M *et al* (2009) Astrocytic necrosis is induced by anti-aquaporin-4 antibody-positive serum. *Neuroreport* **20**:508–512.
21. Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T *et al* (2010) Anti-aquaporin-4 antibody induces astrocytic cytotoxicity

© 2011 The Authors; Brain Pathology © 2011 International Society of Neuropathology

in the absence of CNS antigen-specific T cells. *Biochem Biophys Res Commun* **394**:205–210.
22. Kira J (2010) Neuromyelitis optica and opticospinal multiple sclerosis: mechanisms and pathogenesis. *Pathophysiology* **18**:69–79.
23. Kishimoto R, Yabe I, Niino M, Sato K, Tsuji S, Kikuchi S, Sasaki H (2008) Balo's concentric sclerosislike lesion in the brainstem of a multiple sclerosis patient. *J Neurol* **255**:760–761.
24. Kobayashi Z, Tsuchiya K, Uchihara T, Nakamura A, Haga C, Yokota O *et al* (2009) Intractable hiccup caused by medulla oblongata lesions: a study of an autopsy patient with possible neuromyelitis optica. *J Neurol Sci* **285**:241–245.
25. Kuroiwa Y (1985) Neuromyelitis optica (Devic's disease, Devic's syndrome. In: *Handbook of Clinical Neurology Vol. 3: Demyelinating Diseases*, JC Koetsier (ed.), pp. 397–408. Elsevier Science Publishers: Amsterdam.
26. Lassmann H, Raine CS, Antel J, Prineas JW (1998) Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. *J Neuroimmunol* **86**:213–217.
27. Lee TS, Eid T, Mane S, Kim JH, Spencer DD, Ottersen OP, de Lanerolle NC (2004) Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathol* **108**:493–502.
28. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K *et al* (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* **364**:2106–2112.
29. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* **202**:473–477.
30. Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM *et al* (2002) A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* **125**:1450–1461.
31. Matsuoka T, Matsushita T, Kawano Y, Osoegawa M, Ochi H, Ishizu T *et al* (2007) Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. *Brain* **130**:1206–1223.
32. Matsuoka T, Matsushita T, Osoegawa M, Ochi H, Kawano Y, Mihara F *et al* (2008) Heterogeneity and continuum of multiple sclerosis in Japanese according to magnetic resonance imaging findings. *J Neurol Sci* **266**:115–125.
33. Matsuoka T, Suzuki SO, Iwaki T, Tabira T, Ordinario AT, Kira J (2010) Aquaporin-4 astrocytopathy in Baló's disease. *Acta Neuropathol* **120**:651–660.
34. Matsushita T, Isobe N, Matsuoka T, Ishizu T, Kawano Y, Yoshiura T *et al* (2009) Extensive vasogenic edema of anti-aquaporin-4 antibody-related brain lesions. *Mult Scler* **15**:1113–1117.
35. Matsushita T, Isobe N, Matsuoka T, Shi N, Kawano Y, Wu XM *et al* (2009) Aquaporin-4 autoimmune syndrome and anti-aquaporin-4 antibody-negative opticospinal multiple sclerosis in Japanese. *Mult Scler* **15**:834–847.
36. Matsushita T, Isobe N, Piao H, Matsuoka T, Ishizu T, Doi H *et al* (2010) Reappraisal of brain MRI features in patients with multiple sclerosis and neuromyelitis optica according to anti-aquaporin-4 antibody status. *J Neurol Sci* **291**:37–43.
37. Minohara M, Matsuoka T, Li W, Osoegawa M, Ishizu T, Ohyagi Y, Kira J (2006) Upregulation of myeloperoxidase in patients with opticospinal multiple sclerosis: positive correlation with disease severity. *J Neuroimmunol* **178**:156–160.
38. Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S *et al* (2007) Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain* **130**:1224–1234.
39. Nakashima I, Fujihara K, Miyazawa I, Misu T, Narikawa K, Nakamura M *et al* (2006) Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. *J Neurol Neurosurg Psychiatry* **77**:1073–1075.
40. Okinaka S, Tsubaki T, Kuroiwa Y, Toyokura Y, Imamura Y (1958) Multiple sclerosis and allied diseases in Japan; clinical characteristics. *Neurology* **8**:756–763.
41 Osoegawa M, Kira J, Fukazawa T, Fujihara K, Kikuchi S, Matsui M *et al* (2009) Temporal changes and geographical differences in multiple sclerosis phenotypes in Japanese: nationwide survey results over 30 years. *Mult Scler* **15**:159–173.
42. Paul F, Jarius S, Aktas O, Bluthner M, Bauer O, Appelhans H *et al* (2007) Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. *PLoS Med* **4**:e133.
43. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG (2006) Brain abnormalities in neuromyelitis optica. *Arch Neurol* **63**:390–396.
44. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC *et al* (1983) New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* **13**:227–231.
45. Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W *et al* (2007) Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain* **130**:1194–1205.
46. Saadoun S, Waters P, Bell BA, Vincent A, Verkman AS, Papadopoulos MC (2010) Intra-cerebral injection of neuromyelitis optica immunoglobulin G and human complement produces neuromyelitis optica lesions in mice. *Brain* **133**:349–361.
47. Sabater L, Giralt A, Boronat A, Hankiewicz K, Blanco Y, Llufriu S *et al* (2009) Cytotoxic effect of neuromyelitis optica antibody (NMO-IgG) to astrocytes: an *in vitro* study. *J Neuroimmunol* **215**:31–35.
48. Sharma R, Fischer MT, Bauer J, Felts PA, Smith KJ, Misu T *et al* (2010) Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. *Acta Neuropathol* **120**:223–236.
49. Su JJ, Osoegawa M, Matsuoka T, Minohara M, Tanaka M, Ishizu T *et al* (2006) Upregulation of vascular growth factors in multiple sclerosis: correlation with MRI findings. *J Neurol Sci* **243**:21–30.
50. Tabira T, Tateishi J (1982) Neuropathological features of MS in Japan. In: *Multiple Sclerosis East and West*, Y Kuroiwa, LT Kurland (eds), pp. 273–295. Kyushu University Press: Fukuoka.
51. Viegas S, Weir A, Esiri M, Kuker W, Waters P, Leite MI *et al* (2009) Symptomatic, radiological and pathological involvement of the hypothalamus in neuromyelitis optica. *J Neurol Neurosurg Psychiatry* **80**:679–682.
52. Vincent T, Saikali P, Cayrol R, Roth AD, Bar-Or A, Prat A, Antel JP (2008) Functional consequences of neuromyelitis optica-IgG astrocyte interactions on blood-brain barrier permeability and granulocyte recruitment. *J Immunol* **181**:5730–5737.
53. Wang CD, Zhang KN, Wu XM, Gang H, Xie XF, Qu XH, Xiong YQ (2008) Balo's disease showing benign clinical course and co-existence with multiple sclerosis-like lesions in Chinese. *Mult Scler* **14**:418–424.
54. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG (1999) The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology* **53**:1107–1114.
55. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG (2006) Revised diagnostic criteria for neuromyelitis optica. *Neurology* **66**:1485–1489.
56. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG (2007) The spectrum of neuromyelitis optica. *Lancet Neurol* **6**:805–815.
57. Yanagawa K, Kawachi I, Toyoshima Y, Yokoseki A, Arakawa M, Hasegawa A *et al* (2009) Pathologic and immunologic profiles of a limited form of neuromyelitis optica with myelitis. *Neurology* **73**:1628–1637.

© 2011 The Authors; Brain Pathology © 2011 International Society of Neuropathology