neutrophils and eosinophils that then facilitate tissue destruction. The observation that the anti-AQP4 antibodies so far examined by immunofluorescence assays are all from the IgG1 subclass [7] and can efficiently fix complement is compatible with such a hypothesis. Here, demyelination is secondary to destruction of astrocytes, which is supposed to be fundamentally distinct from the primary demyelinating mechanism executed by myelin antigen-specific T cells and anti-myelin autoantibodies in MS.

7.2. Concerns about the proposed mechanism of NMO

I have several concerns surrounding the above-mentioned hypothesis based on the anti-AQP4 antibody. First, in the presence of high titers of anti-AQP4 antibodies, some patients remain in remission [7], and there are cases where patients who harbor the anti-AQP4 antibody do not show signs of NMO for a long time [79]. Because AQP4 is present in astrocyte foot processes behind the BBB, additional factors that disrupt the BBB and render the antibody able to enter the CNS across the BBB may be required to initiate relapse. In addition, the fact that anti-AQP4 antibody titers [6,7,38,39] do not strictly correlate with the occurrence of relapse in most studies to date further supports the prerequisite for some additional factor to induce relapse. Indeed, in animal models, myelin antigen-specific T cells are required for the anti-AQP4 antibody to cause extensive lesions in vivo in the CNS [56,57]. Our observation that in anti-AQP4 antibody-positive NMO patients' peripheral blood T cells reactive to major myelin proteins showed intra- and inter-molecular epitope spreading [62] suggests that T cells are already stimulated with myelin antigens in vivo in these patients. Second, AQP4 is present in the retina, distal collecting tubules, gastric mucosa, muscle and lung, and NMO-IgG binds to these structures [4,78]; however, no impairments in these organs have been observed to date. In particular, although Müller cells, which are equivalent to astrocytes, abundantly express AQP4 in the endfeet adjacent to blood vessels in the retina, no severe inflammation has ever been reported in anti-AQP4 antibody-positive NMO patients, suggesting that the presence of the complement-fixing anti-AQP4 antibody is not sufficient to produce tissue damage. Moreover, AQP4 expression is ubiquitous throughout the CNS, although its expression level varies, being high in the gray matter of the spinal cord [11,22]. Cerebral gray matter and the cerebellum abundantly express AQP4 [11,22]; however, these sites are seldom involved in NMO [80]. Such a ubiquitous presence of AQP4 cannot explain the selectiveness of lesion distribution, namely in the optic nerves and spinal cord. Third, the deposited immunoglobulins in postmortem NMO lesions are mainly IgM [20] while the anti-AQP4 antibodies described are all IgG. We observed that some NMO lesions show perivascular deposition of complement and IgG in acute lesions, while no AQP4 loss is found [11], suggesting that perivascular complement and IgG deposition does not strictly correlate with AQP4 loss.

7.3. Mechanism of huge CNS lesions

Anti-AQP4 antibody-positive NMO patients occasionally develop huge brain lesions, such as LESCLs seen in the spinal cord. Such extensive white matter lesions in anti-AQP4 antibody-positive NMO patients demonstrate high signal intensity on apparent diffusion coefficient maps and low or isointensity on diffusion-weighted MRI images, suggesting the nature of the lesions to be vasogenic edema [6,25]. Using magnetic resonance spectroscopy, a high choline peak and a low n-acetyl aspartate peak are observed, compatible with acute demyelination [6,25]. These findings strongly suggest that the nature of the lesions in anti-AQP4 antibody-positive MS patients is inflammatory demyelination with vasogenic edema. However, interestingly, even in such extensive brain lesions, gadolinium enhancement of the lesions is absent or scant [25,81], except for cases complicated with other systemic autoimmune diseases, suggesting largely preserved integrity of the BBB in this condition. Contrarily, Ito et al. [82] reported that multiple patchy enhancing lesions with blurred margins, described as "cloud-like enhancement", are found in 90% of NMO patients with contrast enhancement, suggesting breakdown of the BBB in this condition. Thus, the mechanism underlying lesion formation could be heterogeneous, even among individuals with the anti-AQP4 antibody.

7.4. Our hypothesis

The idea that astrocytopathy induces secondary demyelination or that such a tiny deposition of immunoglobulin and complement around vessels produces huge lesions is ill-defined and requires further clarification. We recently found that connexins, which form gap junction channels between glial cells and between glia and axons, were entirely lost in the AQP4-diminished lesions in Baló's disease, NMO and MS (submitted for publication). Connexin gap junction channels not only appose glial cells but also have a critical role in intercellular communication between glia. Therefore, we hypothesize that once glial connexins are disrupted by either autoantibodies, such as the anti-AQP4 antibody, or T cells in the perivascular areas, dysfunction of glial cell interaction may spread along with neural fibers (Fig. 4A). Connexin loss may cause widespread disruption of intercellular communication while AQP4 loss exacerbates vasogenic edema. AQP4 and connexin astrocytopathy occurs in Baló's disease and a fraction of MS and NMO, culminating in huge CNS lesions (Fig. 4B). Such a hypothesis should be tested by future experimental studies.

Acknowledgments

I would like to thank Drs. Takeshi Matsuoka, Satoshi O Suzuki, Katsuhisa Masaki, Noriko Isobe, Tomomi Yonekawa and Takuya Matsushita for their help with neuropathological and immunological examinations. 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.

References

[1] Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology 1999;53:1107–14.
[2] Kira J. Multiple sclerosis in the Japanese population. Lancet Neurol 2003;2:117–27.
[3] Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004;364:2106–12.
[4] 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;202:473–7.
[5] Nakashima I, Fujihara K, Miyazawa I, Misu T, Narikawa K, Nakamura M, et al. Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. J Neurol Neurosurg Psychiatry 2006;77:1073–5.
[6] Matsuoka T, Matsushita T, Kawano Y, Osoegawa M, Ochi H, Ishizu T, et al. Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. Brain 2007;130:1206–23.
[7] Matsushita T, Isobe N, Matsuoka T, Shi N, Kawano Y, Wu XM, et al. Aquaporin-4 autoimmune syndrome and anti-aquaporin-4 antibody-negative opticospinal multiple sclerosis in Japanese. Mult Scler 2009;15:834–47.
[8] Kuroiwa Y. Concentric sclerosis. In: Koetsier JC, editor. Handbook of clinical neurology: demyelinating disease, 47. Amsterdam: Elsevier; 1985. p. 409–17.
[9] Matsuoka T, Suzuki SO, Iwaki T, Tabira T, Ordinario AT, Kira J. Aquaporin-4 astrocytopathy in Baló's disease. Acta Neuropathol 2010;120:651–60.
[10] Graber JJ, Kister I, Geyer H, Khaund M, Herbert J. Neuromyelitis optica and concentric rings of Baló in the brainstem. Arch Neurol 2009;66:274–5.
[11] Matsuoka T, Suzuki SO, Suenaga T, Iwaki T, Kira J. Reappraisal of aquaporin-4 astrocytopathy in Asian neuromyelitis optica and multiple sclerosis patients. Brain Pathol 2011;21:516–32.
[12] Dević E. Myélite subaiguë compliquée de névrite optique-Autopsie. Congrés Francais de Médecine, 1; 1894. p. 434–9.
[13] Beck GM. Diffuse myelitis with optic neuritis. Brain 1927;50:687–703.
[14] Balser BH. Neuromyelitis optica. Brain 1936;59:353–65.

[15] Stansbury FC. Neuromyelitis optica (Devic's disease); presentation of five cases, with pathologic study, and review of literature. Arch Ophthalmol 1949;42: 292–335.
[16] Okinaka S, Tsubaki T, Kuroiwa Y, Toyokura Y, Imamura Y. Multiple sclerosis and allied diseases in Japan; clinical characteristics. Neurology 1958;8:756–63.
[17] Hung TP, Landsborough D, His MS. Multiple sclerosis amongst Chinese in Taiwan. J Neurol Sci 1976;27:459–84.
[18] Tabira T, Tateishi J. Neuropathological features of MS in Japan. In: Kuroiwa Y, Kurland LT, editors. Multiple sclerosis East and West. Fukuoka: Kyushu University Press; 1982. p. 273–95.
[19] Ikuta F, Koga M, Takeda S, Ohama E, Takeshita I, Ogawa H. Comparison of MS pathology between 70 American and 75 Japanese autopsy cases. In: Kuroiwa Y, Kurland LT, editors. Multiple sclerosis East and West. Fukuoka: Kyushu University Press; 1982. p. 297–306.
[20] Lucchinetti CF, Mandler RN, McGavern D, Brück W, Gleich G, Ransohoff RM, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 2002;125:1450–61.
[21] Ishizu T, Osoegawa M, Mei FJ, Kikuchi H, Tanaka M, Takakura Y, et al. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain 2005;128: 988–1002.
[22] Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 2007;130:1224–34.
[23] Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Brück W, et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 2007;130:1194–205.
[24] Kobayashi Z, Tsuchiya K, Uchihara T, Nakamura A, Haga C, Yokota O, et al. Intractable hiccup caused by medulla oblongata lesions: a study of an autopsy patient with possible neuromyelitis optica. J Neurol Sci 2009;285:241–5.
[25] Matsushita T, Isobe N, Matsuoka T, Ishizu T, Kawano Y, Yoshiura T, et al. Extensive vasogenic edema of anti-aquaporin-4 antibody-related brain lesions. Mult Scler 2009;15:1113–7.
[26] Kira J. Astrocytopathy in Balo's disease. Mult Scler 2011;17:771–9.
[27] Paul F, Jarius S, Aktas O, Bluthner M, Bauer O, Appelhans H, et al. Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. PLoS Med 2007;4:e133.
[28] Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain abnormalities in neuromyelitis optica. Arch Neurol 2006;63:390–6.
[29] Jarius S, Franciotta D, Bergamaschi R, Wright H, Littleton E, Palace J, et al. NMO-IgG in the diagnosis of neuromyelitis optica. Neurology 2007;68:1076–7.
[30] Fazio R, Malosio ML, Lampasona V, De Feo D, Privitera D, Marnetto F, et al. Antiaquaporin 4 antibodies detection by different techniques in neuromyelitis optica patients. Mult Scler 2009;15:1153–63.
[31] Cabrera-Gomez JA, Bonnan M, Gonzalez-Quevedo A, Saiz-Hinarejos A, Marignier R, Olindo S, et al. Neuromyelitis optica positive antibodies confer a worse course in relapsing-neuromyelitis optica in Cuba and French West Indies. Mult Scler 2009;15:828–33.
[32] Cree BA, Reich DE, Khan O, De Jager PL, Nakashima I, Takahashi T, et al. Modification of multiple sclerosis phenotypes by African ancestry at HLA. Arch Neurol 2009;66:226–33.
[33] Takahashi T, Fujihara K, Nakashima I, Misu T, Miyazawa I, Nakamura M, et al. Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre. Brain 2007;130:1235–43.
[34] Tanaka K, Tani T, Tanaka M, Saida T, Idezuka J, Yamazaki M, et al. Anti-aquaporin 4 antibody in selected Japanese multiple sclerosis patients with long spinal cord lesions. Mult Scler 2007;13:850–5.
[35] Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120:2059–69.
[36] Osoegawa M, Kira J, Fukazawa T, Fujihara K, Kikuchi S, Matsui M, et al. Temporal changes and geographical differences in multiple sclerosis phenotypes in Japanese: nationwide survey results over 30 years. Mult Scler 2009;15:159–73.
[37] Ishizu T, Kira J, Osoegawa M, Fukazawa T, Kikuchi S, Fujihara K, et al. Heterogeneity and continuum of multiple sclerosis phenotypes in Japanese according to the results of the fourth nationwide survey. J Neurol Sci 2009;280:22–8.
[38] Adoni T, Lino AM, da Gama PD, Apostolos-Pereira SL, Marchiori PE, Kok F, et al. Recurrent neuromyelitis optica in Brazilian patients: clinical, immunological, and neuroimaging characteristics. Mult Scler 2009;16:81–6.
[39] Hinson SR, McKeon A, Fryer JP, Apiwattanakul M, Lennon VA, Pittock SJ. Prediction of neuromyelitis optica attack severity by quantitation of complement-mediated injury to aquaporin-4-expressing cells. Arch Neurol 2009;66:1164–7.
[40] Weinshenker BG, Wingerchuk DM, Vukusic S, Linbo L, Pittock SJ, Lucchinetti CF, et al. Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. Ann Neurol 2006;59:566–9.
[41] Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, et al. Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 2007;204:2363–72.
[42] Isobe N, Yonekawa T, Matsushita T, Kawano Y, Masaki K, Yoshimura S, et al. Distinct features of neuromyelitis optica according to anti-aquaporin-4 antibody IgG subclass. American Neurological Association 136th Annual Meeting; 2011.
[43] Mader S, Lutterotti A, Di Pauli F, Kuenz B, Schanda K, Aboul-Enein F, et al. Patterns of antibody binding to aquaporin-4 isoforms in neuromyelitis optica. PLoS One 2010;5:e10455.
[44] Jarius S, Aboul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. Brain 2008;131: 3072–80.
[45] Köller H, Neuen-Jacob E, Saleh A, Kieseier BC, Jander S, Hartung HP. A patient with a benign course of neuromyelitis optica (Devic's syndrome) over 12 years: MRI follow up and histological findings. J Neurol 2006;253:819–20.
[46] Bergamaschi R, Jarius S, Robotti M, Pichiecchio A, Wildemann B, Meola G. Two cases of benign neuromyelitis optica in patients with celiac disease. J Neurol 2009;256:2097–9.
[47] McKeon A, Lennon VA, Jacob A, Matiello M, Lucchinetti CF, Kale N, et al. Coexistence of myasthenia gravis and serological markers of neurological autoimmunity in neuromyelitis optica. Muscle Nerve 2009;39:87–90.
[48] Uzawa A, Mori M, Iwai Y, Kobayashi M, Hayakawa S, Kawaguchi N, et al. Association of anti-aquaporin-4 antibody-positive neuromyelitis optica with myasthenia gravis. J Neurol Sci 2009;287:105–7.
[49] Kim SM, Waters P, Vincent A, Kim SY, Kim HJ, Hong YH, et al. Sjogren's syndrome myelopathy: spinal cord involvement in Sjogren's syndrome might be a manifestation of neuromyelitis optica. Mult Scler 2009;15:1062–8.
[50] Min JH, Kim HJ, Kim BJ, Lee KW, Sunwoo IN, Kim SM, et al. Brain abnormalities in Sjogren syndrome with recurrent CNS manifestations: association with neuromyelitis optica. Mult Scler 2009;15:1069–76.
[51] Vincent T, Saikali P, Cayrol R, Roth AD, Bar-Or A, Prat A, et al. Functional consequences of neuromyelitis optica-IgG astrocyte interactions on blood–brain barrier permeability and granulocyte recruitment. J Immunol 2008;181:5730–7.
[52] Sabater L, Giralt A, Boronat A, Hankiewicz K, Blanco Y, Llufriu S, et al. Cytotoxic effect of neuromyelitis optica antibody (NMO-IgG) to astrocytes: an in vitro study. J Neuroimmunol 2009;215:31–5.
[53] Kinoshita M, Nakatsuji Y, Moriya M, Okuno T, Kumanogoh A, Nakano M, et al. Astrocytic necrosis is induced by anti-aquaporin-4 antibody-positive serum. Neuroreport 2009;20:508–12.
[54] Nicchia GP, Mastrototaro M, Rossi A, Pisani F, Tortorella C, Ruggieri M, et al. Aquaporin-4 orthogonal arrays of particles are the target for neuromyelitis optica autoantibodies. Glia 2009;57:1363–73.
[55] Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T, et al. Neuromyelitis optica: passive transfer to rats by human immunoglobulin. Biochem Biophys Res Commun 2009;386:623–7.
[56] Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C, et al. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. Ann Neurol 2009;66:617–29.
[57] Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M, et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. Ann Neurol 2009;66: 630–43.
[58] Saadoun S, Waters P, Bell BA, Vincent A, Verkman AS, Papadopoulos MC. Intracerebral injection of neuromyelitis optica immunoglobulin G and human complement produces neuromyelitis optica lesions in mice. Brain 2010;133:349–61.
[59] Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T, et al. Anti-aquaporin-4 antibody induces astrocytic cytotoxicity in the absence of CNS antigen-specific T cells. Biochem Biophys Res Commun 2010;394:205–10.
[60] Kalluri SR, Rothhammer V, Staszewski O, Srivastava R, Petermann F, Prinz M, et al. Functional characterization of aquaporin-4 specific T cells: towards a model for neuromyelitis optica. PLoS One 2011;6:e16083.
[61] Minohara M, Ochi H, Matsushita S, Irie A, Nishimura Y, Kira J. Differences between T-cell reactivities to major myelin protein-derived peptides in opticospinal and conventional forms of multiple sclerosis and healthy controls. Tissue Antigens 2001;57:447–56.
[62] Yonekawa T, Matsushita T, Minohara M, Isobe N, Masaki K, Yoshimura S, et al. T cell reactivities to myelin protein-derived peptides in neuromyelitis optica patients with anti-aquaporin-4 antibody. Neurology Asia 2011;16:139–42.
[63] Ochi H, Wu XM, Osoegawa M, Horiuchi I, Minohara M, Murai H, et al. Tc1/Tc2 and Th1/Th2 balance in Asian and Western types of multiple sclerosis, HTLV-I-associated myelopathy/tropical spastic paraparesis and hyperIgEaemic myelitis. J Neuroimmunol 2001;119:297–305.
[64] Wu XM, Osoegawa M, Yamasaki K, Kawano Y, Ochi H, Horiuchi I, et al. Flow cytometric differentiation of Asian and Western types of multiple sclerosis, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and hyperIgEaemic myelitis by analyses of memory CD4 positive T cell subsets and NK cell subsets. J Neurol Sci 2000;177:24–31.
[65] Tanaka M, Matsushita T, Tateishi T, Ochi H, Kawano Y, Mei FJ, et al. Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. Neurology 2008;71:974–81.
[66] Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821–52.
[67] Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–8.
[68] Minohara M, Matsuoka T, Li W, Osoegawa M, Ishizu T, Ohyagi Y, et al. Upregulation of myeloperoxidase in patients with opticospinal multiple sclerosis: positive correlation with disease severity. J Neuroimmunol 2006;178:156–60.
[69] Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, et al. Human TH17 lymphocytes promote blood–brain barrier disruption and central nervous system inflammation. Nat Med 2007;13:1173–5.
[70] Ryu S, Lee JH, Kim SI. IL-17 increased the production of vascular endothelial growth factor in rheumatoid arthritis synoviocytes. Clin Rheumatol 2006;25: 16–20.
[71] Su JJ, Osoegawa M, Matsuoka T, Minohara M, Tanaka M, Ishizu T, et al. Upregulation of vascular growth factors in multiple sclerosis: correlation with MRI findings. J Neurol Sci 2006;243:21–30.
[72] Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 2004;18:1291–3.

[73] Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med 2000;6:159–63.
[74] Sellner J, Hemmer B, Muhlau M. The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes. J Autoimmun 2010;34:371–9.
[75] Doi H, Matsushita T, Isobe N, Matsuoka T, Minohara M, Ochi H, et al. Hypercomplementemia at relapse in patients with anti-aquaporin-4 antibody. Mult Scler 2009;15:304–10.
[76] Li W, Minohara M, Su JJ, Matsuoka T, Osoegawa M, Ishizu T, et al. Helicobacter pylori is a potential protective factor against conventional type multiple sclerosis in the Japanese population. J Neuroimmunol 2007;182:232–5.
[77] Li W, Minohara M, Piao H, Matsushita T, Masaki K, Matsuoka T, et al. Association of anti-*Helicobacter pylori* neutrophil-activating protein antibody response with anti-aquaporin-4 autoimmunity in Japanese patients with multiple sclerosis and neuromyelitis optica. Mult Scler 2009;15:1411–21.
[78] Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol 2007;6:805–15.
[79] Pittock SJ, Lennon VA. Aquaporin-4 autoantibodies in a paraneoplastic context. Arch Neurol 2008;65:629–32.
[80] Almekhlafi MA, Clark AW, Lucchinetti CF, Zhang Y, Power C, Bell RB. Neuromyelitis optica with extensive active brain involvement: an autopsy study. Arch Neurol 2011;68:508–12.
[81] Saiki S, Ueno Y, Moritani T, Sato T, Sekine T, Kawajiri S, et al. Extensive hemispheric lesions with radiological evidence of blood–brain barrier integrity in a patient with neuromyelitis optica. J Neurol Sci 2009;284:217–9.
[82] Ito S, Mori M, Makino T, Hayakawa S, Kuwabara S. "Cloud-like enhancement" is a magnetic resonance imaging abnormality specific to neuromyelitis optica. Ann Neurol 2009;66:425–8.

MULTIPLE SCLEROSIS JOURNAL | MSJ

Multiple Sclerosis Journal
17(7) 771–779

DOI: 10.1177/1352458511400475
msj.sagepub.com
SAGE

Astrocytopathy in Baló's disease

Jun-ichi Kira

Abstract

Baló's disease is characterized by alternating rings of demyelination and preserved myelin. As additional multiple sclerosis (MS)-like lesions often coexist in Baló's cases, Baló's disease is regarded as a variant of MS. In demyelinated areas, many hypertrophic astrocytes are present in close contact with oligodendrocytes, which often show apoptotic features. In the outermost layer of preserved myelin, stress proteins involved in tissue preconditioning are abundant in oligodendrocytes. The peri-plaque perimeter is thus assumed resistant to subsequent attack, thereby leaving a layer of preserved myelin. In some cases, Baló's concentric rings develop step by step in a centrifugal direction, whereas many other cases show simultaneous enhancement of multiple rings. Therefore tissue preconditioning and successive ring formation does not fully describe the mechanism of the disease. We recently reported that in four Filipino Baló's patients, aquaporin-4 (AQP4) was extensively lost in glial fibrillary acidic protein-positive hypertrophic astrocytes, both in demyelinated and myelinated layers of all actively demyelinating lesions. None of six further patients with MRI-confirmed Baló's disease were seropositive for anti-AQP4 antibody. I propose that AQP4 astrocytopathy, in the absence of anti-AQP4 antibody, is characteristic of Baló's disease. This hypothesis should be tested in future experimental studies.

Keywords

aquaporin-4, astrocytopathy, Baló's disease, concentric lesions, multiple sclerosis (MS), neuromyelitis optica (NMO)

Date received: 26th September 2010; revised: 8th December 2010; accepted: 17th January 2011

Introduction and methods

Baló's disease was first described by Marburg[1] more than a century ago, under the name 'encephalitis periaxialis scleroticans'. Baló, a Hungarian neuropathologist, was the first to stress a concentric pattern of lesions, and he named the disease 'encephalitis periaxialis concentrica', based on the observation that 'the white matter of the brain is destroyed in concentric layers in a manner that leaves the axis cylinders intact'.[2] The condition has since been named after him.

Baló's disease is diagnosed histologically, using autopsied tissues, by the presence of alternating layers of demyelination and relatively preserved myelin. Historically, the disease was very rarely reported; however, since the introduction of magnetic resonance imaging (MRI) into clinical practice, the number of reported cases with Baló-like concentric lesions has increased. Such neuroimaging studies, together with recent immunohistochemical analyses, have given important insight into the pathophysiology of Baló's disease. Although the peculiar concentric nature of the lesions has attracted a lot of attention, the mechanism producing this pathology remains to be elucidated. We recently reported extensive aquaporin-4 (AQP4) loss in Baló's disease lesions and pointed out the importance of AQP4 astrocytopathy in the absence of anti-AQP4 antibodies.[3] Here, I have conducted a literature search on Baló's disease using PubMed and reviewed recent progress in research on Baló's disease. I propose a novel mechanism for concentric demyelinating ring formation.

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Corresponding author:
Jun-ichi Kira MD, PhD, Professor and Chairman, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan
Email: kira@neuro.med.kyushu-u.ac.jp

Clinical findings of Baló's disease

Baló's disease is rare in White populations but is relatively frequently reported in certain Asian populations, such as Filipinos, southern Han Chinese and Taiwanese.[4] The disease most commonly shows an acute onset, although some cases have a subacute onset. Initial symptoms are usually mental and behavioral changes and these symptoms progress into major deficits within a few months, presenting paralysis, hyperreflexia, seizure, dysphagia, sphincter impairment, and akinetic mutism. The severity of symptoms is in accord with lesion expansion determined by MRI. Without treatment, the disease culminates in death or severe disability. Cerebrospinal fluid (CSF) is normal or has mild inflammatory changes.[4,5] Oligoclonal bands (OBs) are usually absent in CSF, while some recent cases, demonstrating a transition to multiple sclerosis (MS), were reported to have OBs.[6–10]

A monophasic course and poor prognosis were previously thought to be common; however, MRI has made clinical diagnosis of Baló's concentric rings possible, and early intervention with high-dose corticosteroids and other immunotherapies has markedly improved its prognosis.[11] Cases showing spontaneous recovery have even been reported.[6] Some Baló's disease cases have recovered from the initial insult, but have had a relapsing course and demonstrated the coexistence of Baló-like concentric lesions and MS-like lesions.[11] Also, some cases initially diagnosed as MS have later developed Baló's concentric lesions, as determined by MRI.[7,12] Since the clinical introduction of MRI, a considerable number of reports have described a transition of these two conditions.[8–12] In these articles, some cases have been reported as Baló's disease while others have been described as MS with concentric lesions.[8–12] Hence, it is difficult to completely differentiate Baló's disease from MS with concentric aspects, and some authors regard Baló's disease as a variant of MS.[11]

Pathological findings of Baló's disease

Early pathological studies revealed that the most commonly affected site is the cerebral white matter. The pons, cerebellar white matter and basal ganglia were also affected in some autopsied cases.[5,13] Cortical gray matter and subcortical U fibers are usually spared, as distinct from MS.[4,5,14] The spinal cord was long considered to be unaffected in Baló's disease; however, recent MRI-diagnosed or pathologically proven cases have shown this to be untrue.[15,16] Concentric rings are typically parallel rings with a 'storm center',[5] but there are cases showing various other patterns, such as distorted rings, a rosette or carnation pattern, a mosaic pattern, and parallel bars.[5] Demyelinated layers are consistently wider than myelinated ones.[14] Within the demyelinated bands, the inner border is sharp whereas the outer border is blurred and gradually transitions to the white matter at the outermost edge, which appears normal.[14]

Moore et al.[15] noted in a Baló's case that the bands of intact myelin comprised mainly remyelinated fibers, and they proposed the possibility of remyelination for the myelinated layers. However, Yao et al.[17] and others[4] reported that in the demyelinated areas, where Bodian staining showed axons to be relatively preserved, there were no thinly myelinated fibers, indicating that remyelination is not responsible for the occurrence of the myelinated rings. Yao et al.[17] found that the number of oligodendrocytes was markedly reduced in the demyelinated layers, and, after long disease duration, oligodendrocyte numbers were considerably decreased, even in the relatively preserved myelin layers. These authors also observed that there were many bizarrely shaped giant astrocytes that stained with glial fibrillary acidic protein (GFAP) in the demyelinated as well as the myelinated areas, which closely associated with oligodendrocytes in the demyelinated areas.[17]

Lucchinetti et al.[18] proposed four basic demyelinating patterns of MS lesions. Among them, the so-called pattern III lesions show a characteristic disturbance of oligodendroglia, defined as: distal dying back oligodendrogliopathy with oligodendroglia apoptosis. In this condition, apoptotic nuclear changes in oligodendrocytes and preferential loss of myelin-associated glycoprotein and cyclic-nucleotide phosphodiesterase are characteristic. Lucchinetti et al.[18] described 8 of 22 MS cases with pattern III lesions showing concentric layering of myelinated and demyelinated tissues. Most of these cases had an acute disease course of less than 8 weeks before biopsy or autopsy, while longer surviving cases showed a disease consistent with relapsing–remitting MS.[18] These cases might thus be regarded as MS with concentric aspects, while Baló's disease has some histopathological features of pattern III lesions.[19,20]

Features of pattern III lesions can be seen in a variety of white matter pathologies, such as white matter hypoxia/ischemia, progressive multifocal leukoencephalopathy (PML), and cuprizone intoxication, which interferes with cellular energy metabolism.[19] Hypoxia-like tissue injury has, therefore, been proposed to be critical in Baló's disease.[19,20] Stadelmann et al.[20] found high levels of stress proteins that are involved in tissue preconditioning, such as hypoxia-inducible factor 1α and heat-shock protein, in oligodendroglia in the outermost layer of preserved myelin. Based on the neuroprotective effects of these proteins, the

perimeter layer of peri-plaque tissue is assumed to be resistant to subsequent attack, thereby leaving a layer of preserved myelin.[20]

Vascular and inflammatory components also exist in Baló's concentric lesions. Courville[5] described capillary degeneration in the demyelinated layers but relative capillary preservation in the myelin-preserved layers, suggesting impairment of circulation, especially in the demyelinated areas. Additional small lesions, showing perivascular concentric demyelination, can be seen in some cases, further supporting an important role of vascular factors disrupting the blood–brain barrier (BBB).[14] Many foamy macrophages containing myelin debris infiltrate Baló's lesions, being most numerous around vessels and in the demyelinated areas.[17] Small foci of perivascular mononuclear cell cuffing, which included lymphocytes, was observed in partly myelinated layers, as well as in white matter of normal appearance.[17] We revealed that perivascular lymphocyte cuffing consists mainly of T cells and a few B cells in Baló's concentric lesions, but did not observe any vasculocentric deposition of immunoglobulin or complement.[3]

Neuroimaging findings of Baló's disease

Cases with MRI-proven Baló's concentric lesions have repeatedly been reported after the introduction of MRI into clinical practice. Most of these cases had Baló's concentric lesions in the cerebral hemisphere, although the occurrence of Baló's concentric lesions in the brainstem has also been described.[21,22] Chen et al.[23] proposed that Baló's concentric rings do not arise simultaneously, but develop step by step in a centrifugal direction. These authors observed that enhancement of the outer layers relatively devoid of demyelination developed first, and was followed by progressive demyelination occurring at the inner aspect of the enhancement.[23] Thereafter, new enhancement appeared at the periphery while the previously enhanced rings disappeared. Recurrence of this process forms alternating demyelinated and relatively preserved myelin bands.

Such findings seem to fit well with the preconditioning hypothesis based on the pathological observation of upregulated protective factors in the outermost layers, and support a mechanism of tissue preconditioning and outward expansion of the concentric lesions. By contrast, Kastrup et al.[24] reported that contrast enhancement of T2 hyperintense rings developed synchronously rather than successively. Simultaneous multiple layers of ring enhancement or serpentine ring enhancement are described by many other authors.[8–11] Wang et al.[11] concluded that enhancement patterns of Baló's concentric lesions are heterogeneous: synchronous enhancement of most concentric rings, heterogeneous enhancement of different rings, and confined enhancement of the outermost ring, in part depending on the timing of MRI scans. Baló's concentric rings are already present at the time of initial presentation in many MRI-confirmed cases.[8–11] These observations suggest that tissue preconditioning and successive ring formation cannot fully explain the mechanisms of Baló's disease in all cases.

Although some authors have claimed that Baló's lesions show increased diffusion,[9] Wiendl et al.[25] reported diffusion-weighted MRI abnormalities in a case of Baló's disease on the first day that neurological symptoms appeared. They found markedly restricted diffusion on an apparent diffusion coefficient (ADC) map of a very acute lesion, which MRI showed to have developed Baló's concentric lesions at day 14 after the disease onset. Kavanagh et al.[26] also reported alternating bands of restricted and unrestricted diffusion in a case with Baló's disease. Restricted diffusion is seen in a variety of conditions showing cytotoxic edema, such as brain infarction, PML, acute disseminated encephalomyelitis (ADEM), vasculitis, herpes simplex encephalitis, and Creutzfeldt-Jakob disease.[27–31] MS lesions are usually described as showing an increased ADC due to vasogenic edema following BBB damage and acute demyelination by inflammation.[32] However, MS cases demonstrating restricted diffusion in the acute phase are increasingly being reported.[33–37] Some authors noted increased diffusion in the MS lesion center but restricted diffusion in the periphery,[38] while others even described a conversion of restricted diffusion to a vasogenic edema pattern on serial MRI scans.[35] Such restricted diffusion indicates the presence of cytotoxic edema in acute inflammatory demyelinating lesions, including MS and ADEM, which could be produced by myelin and oligodendroglia swelling, energy failure, and ischemia. It is conceivable that very acute lesions of Baló's disease may have restricted diffusion due to similar mechanisms.

Using serial proton magnetic resonance spectroscopy (MRS), Chen[39] reported a decrease of the *N*-acetyl-aspartate (NAA)/creatine (Cr) ratio, an increase of the choline (Cho)/Cr ratio, and emergence of a lactate peak in Baló's concentric lesions, which are consistent with axonal loss, demyelination, and inflammatory cell infiltration, respectively, as seen in autopsied samples.

Coexistence of neuromyelitis optica and Baló's concentric lesions

Graber et al.[22] reported a 29-year-old Afro-Caribbean female with neuromyelitis optica (NMO) presenting Baló's concentric lesions. She had recurrent episodes

of transverse myelitis and acute optic neuritis, and NMO IgG was positive. At the third attack, she developed visual loss and brainstem signs attributable to Baló's concentric lesions in the brainstem. Kreft et al.[16] also described a similar case of a 57-year-old White female who had episodes of transverse myelitis and bilateral optic neuritis, with normal brain MRI and no OBs in the CSF. She later developed Baló's concentric lesions in the cerebral hemisphere. In both cases, plasma exchange was not effective, suggesting factors other than humoral ones were involved.

Pathologically, occurrence of Baló-type concentric lesions in NMO cases has also been reported in early autopsy studies, under the names of acute disseminated sclerosis[40] and concentric lacunar leukoencephalopathy.[41] Itoyama et al.[42] described concentric lamellar demyelinated lesions in Japanese patients with atypical MS; one had bilateral optic neuritis and transverse myelitis, and the other showed transverse myelitis and brainstem signs. Both showed marked CSF pleocytosis exceeding 300 cells/mm^3. The latter had Baló's concentric lesions in the spinal cord, while the former had Baló-like concentric lesions in the optic chiasm. Both cases might be regarded as cases with NMO or NMO spectrum disorder. These findings collectively suggest that Baló's concentric lesions could emerge in NMO.

Aquaporin-4 astrocytopathy in the absence of anti-aquaporin-4 antibodies in Baló's disease

NMO was also thought to be a variant of MS; however, the recent discovery of NMO IgG targeting the AQP4 water-channel protein expressed in astrocyte foot processes suggests that NMO is distinct from MS.[43,44] In NMO, extensive AQP4 and GFAP loss in areas of relatively preserved myelin, together with vasculocentric deposition of IgM, IgG and activated complement, were reported to be characteristic.[45,46] Therefore, in NMO, it is hypothesized that anti-AQP4 IgG1 antibodies bound to AQP4 expressed on the astrocytes fix complement and initiate an inflammatory cascade, and that the primary target is astrocytes, with demyelination occurring secondarily.

Considering the occurrence of Baló's concentric lesions in NMO cases, we immunohistochemically investigated four autopsied Filipino cases with Baló's disease, aiming to clarify AQP4 expression in this condition.[3] We found that in Baló's concentric lesions, AQP4 was extensively lost in both the demyelinated and myelinated layers of all six actively demyelinating lesions. Numerous CD68-positive macrophages that phagocytose myelin debris had infiltrated the lesions, even though the lesions were entirely and strongly stained with GFAP.[3] In the lesions, hypertrophic astrocytes showed intensive staining for GFAP, while AQP4 expression was totally lost (Figure 1). By contrast, in normal-appearing white matter adjacent to the lesions, AQP4 staining was seen in the perivascular astrocyte foot processes. Only in the outer portions of the concentric lesions were there one or two bands showing loss of GFAP and neurofilament in addition to AQP4 loss. These bands are thus considered to be necrotic, and look similar to 'concentric lacunar leukoencephalopathy' (Baló rings in an NMO case).[41] In the demyelinated layers, Bodian staining revealed relative preservation of axons, whereas neurofilament staining was markedly diminished, suggesting axonal impairment, which is in accord with the decreased NAA/Cr ratio determined by MRS.[39] In demyelinated as well as myelinated layers, connexin 43 immunostaining was also lost in Baló's disease while its expression is usually unregulated in chronic astrogliotic scar.[47] Since connexin 43 forms heteromeric gap junctions among astrocytes and oligodendrocytes, widespread disturbance of astrocyte–astrocyte and astrocyte–oligodendrocyte interaction is suggested in Baló's lesions.

We observed perivascular lymphocyte cuffing by CD45RO-positive T cells, but only by a few CD20-positive B cells in Baló's concentric lesions.[3] Interestingly, there was no deposition of immunoglobulins (IgG and IgM) or complement (C3 and C9neo) around vessels.[3] In the normal-appearing white matter adjacent to the outer edge of the lesions,[47] lamellar infiltration of macrophages was seen, while T cells infiltrated distant areas, which is consistent with the early pathological reports describing lymphocyte infiltration in normal-appearing white matter.[17]

In another six patients with MRI-confirmed Baló's concentric lesions,[11] we examined anti-AQP4 antibody in sera by a conventional immunofluorescence method and a more sensitive flow cytometric assay, and found that none of the cases were positive for anti-AQP4 antibody.[47] These findings collectively suggest that AQP4 astrocytopathy in the absence of anti-AQP4 antibody is characteristic of Baló's disease.

Hypothetical mechanisms for the formation of Baló's concentric rings

A variety of mechanisms have been proposed since the original description of the rings. The current dominant hypothesis is distal oligodendrogliopathy and tissue preconditioning.[19,20] Here, initial insult induces oligodendrocyte apoptosis, and in the lesion edges, oligodendrocytes are preconditioned to produce stress proteins, which work protectively at the second insult

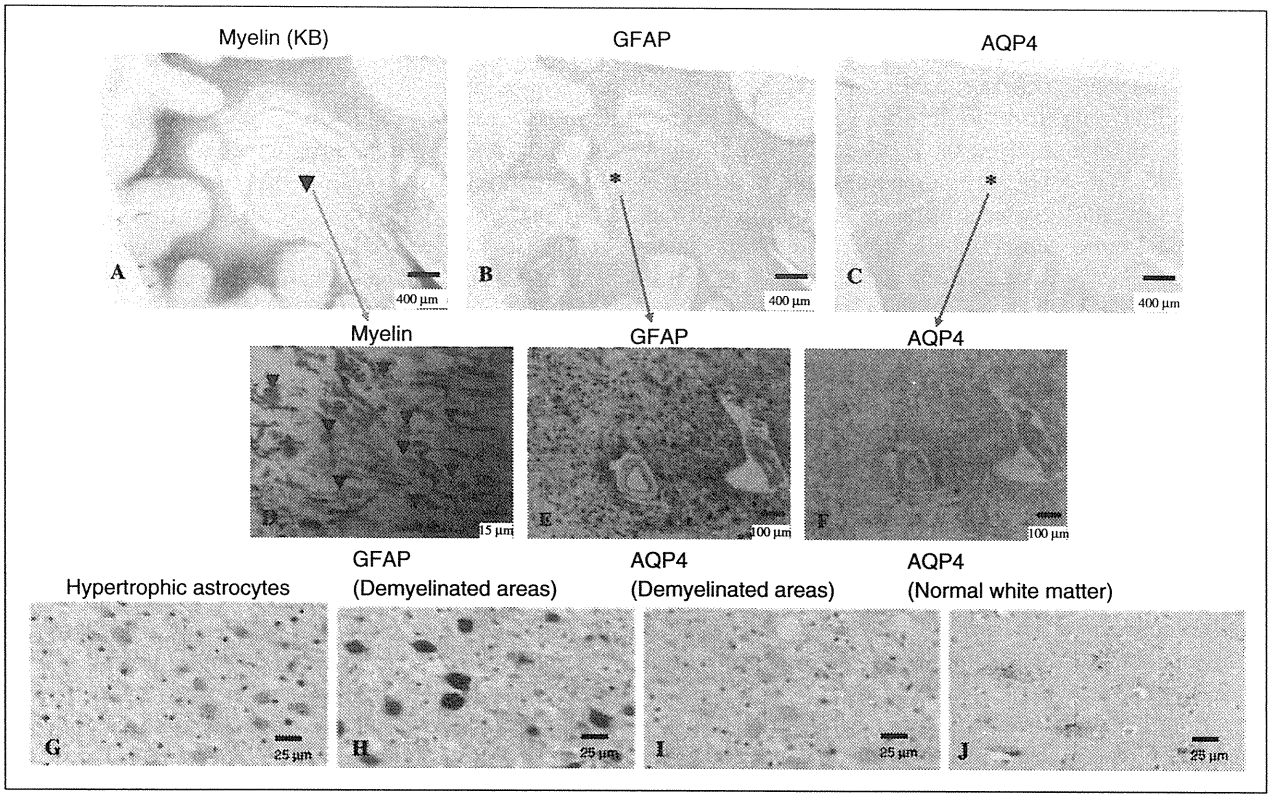

Figure 1. Extensive aquaporin-4 loss in the demyelinated and preserved myelin layers in Baló's concentric sclerosis lesions. (A) The cerebral white matter shows concentric demyelinated lesions. (B) GFAP is expressed in the lesion center, but not in the lamellar necrotic foci at the lesion edge (arrows). (C) AQP4 immunoreactivity is largely lost in the lesion center, with lamellar myelin-staining patterns. (D) Dense infiltration of foamy macrophages phagocytosing myelin debris in the demyelinating layer. (E) Astrocytes strongly express GFAP. (F) Astrocytes lack surface staining for AQP4. (G) Numerous reactive, hypertrophic astrocytes are seen, both in the demyelinated and in the preserved myelin layers. (H) Hypertrophic astrocytes are strongly stained for GFAP. (I) AQP4 is totally lost in hypertrophic astrocytes. (J) Non-reactive astrocytes show AQP4 staining on the cell surface and on their processes in the unaffected white matter, with preserved myelin staining and no inflammatory infiltration. A, D, KB staining; B, E, H, GFAP immunohistochemistry (IHC); C, F, I, J, AQP4 IHC. AQP4 = aquaporin-4; GFAP = glial fibrillary acidic protein; KB = Klüver-Barrera staining. Reproduced in part from Matsuoka et al. *Acta Neuropathologica* 2010; 120: 651–660, with permission.

(Figures 2 and 3). Thereby, concentric rings are formed step by step. However, as mentioned above, Baló's disease frequently shows a concentric lamellar pattern at the time of initial presentation on MRI. Apparently, successive formation of concentric rings by alternating layers of apoptotic oligodendrocytes and preconditioned oligodendrocytes cannot account for the simultaneous emergence of concentric rings in Baló's disease. Extensive AQP4 loss in the demyelinated as well as in the myelinated layers of Baló's concentric rings prompted us to consider astrocytopathy to be upstream of oligodendrogliopathy. Previously reported close contact of hypertrophic astrocytes with oligodendrocytes in the demyelinated layers of Baló's lesions may suggest a possibility that abnormal interaction between these two cell types may lead to oligodendroglial damage followed by extensive demyelination.

At present, we do not know the mechanism of AQP4 loss in the hypertrophic astrocytes in Baló's lesions. In NMO, antibody-mediated killing of astrocytes is proposed. In a rat model of autoimmune encephalomyelitis (EAE), induced by the transfer of myelin basic protein (MBP)-specific T cells, IgG containing anti-AQP4 antibody from IgG-seropositive NMO patients reproduces astrocyte loss in vivo.[48–50] However, no disease or neuropathological CNS alterations were observed when AQP4 antibody was injected into young rats with a leaky BBB, or after transfer of non-encephalitogenic T cells.[50] Thus, anti-AQP4 antibody is pathogenic to the CNS in an inflammatory state. By contrast, such a mechanism cannot be operating in Baló's disease, because there exists neither vasculocentric deposition of immunoglobulin and complement in the lesions nor anti-AQP4 antibody in sera. Since T-cell infiltration

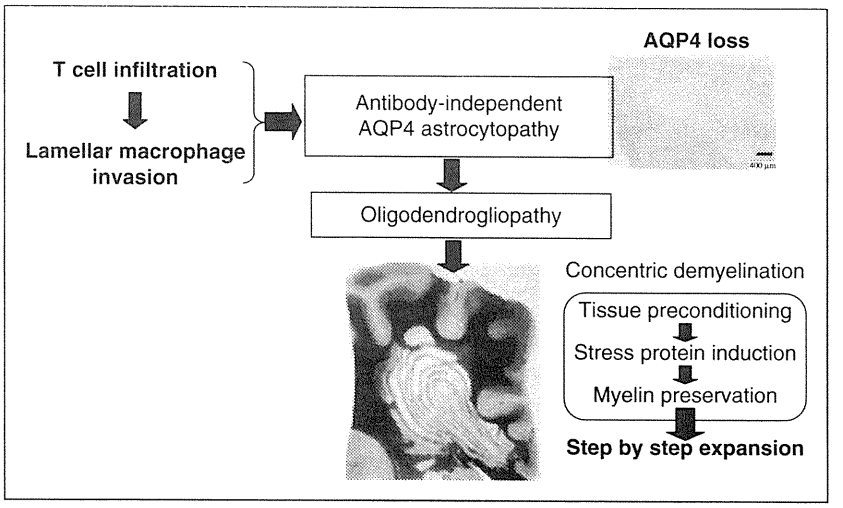

Figure 2. Hypothetical cascade of events in Baló's disease.

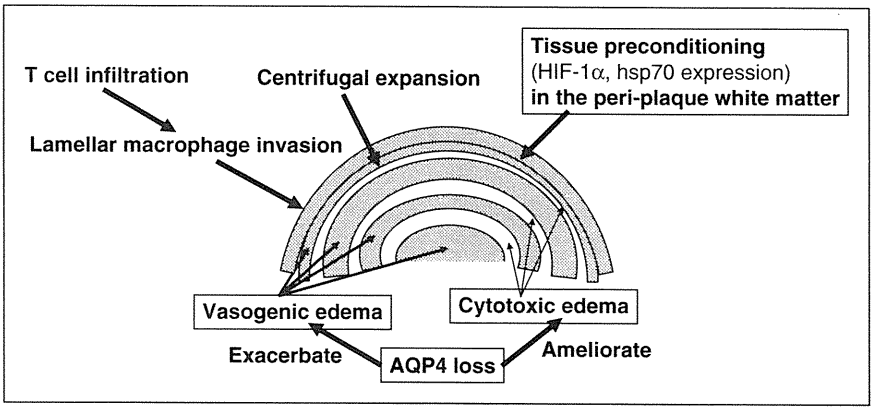

Figure 3. Hypothetical mechanisms for Baló's concentric ring formation.

and macrophage invasion appear to herald development of astrocytopathy and oligodendrogliopathy/demyelination, these immunocytes may induce down-modulation of AQP4 through the actions of inflammatory products. Alternatively, because AQP4 is down-regulated in hypoxic conditions and in the ischemic core at the acute stage,[51–54] vessel obliteration or mitochondrial impairment associated with heavy lymphocytic inflammation may lead to tissue hypoxia, which, in turn, may induce AQP4 down-regulation. The notion that hypoxia-like tissue injury is operative in Baló's concentric sclerosis[19] further supports such a mechanism.

In Baló's disease, very early lesions could be cytotoxic edema, and alternating layers of cytotoxic and vasogenic edema may develop in association with lamellar infiltration of inflammatory cells, which is inferred by concentric or serpentine enhancement of the lesions with contrast media and by alternating bands of increased and restricted diffusion on ADC maps. In AQP4 knockout mice, a prolongation of vasogenic edema,[55] but a decrease in the level of cytotoxic edema[56] was noted and EAE was markedly attenuated.[57] Thus, AQP4 loss is not necessarily harmful in CNS inflammatory conditions. Since AQP4 loss is widespread in entire lesions in Baló's cases, such a loss may potentiate tissue destruction through prolongation of vasogenic edema on one hand, whereas it may ameliorate tissue damage via reduction of cytotoxic edema on the other, resulting in alternating bands of demyelination and preserved myelin. In anti-AQP4 antibody-positive NMO cases, antibody-mediated loss of AQP4 may exert similar influence, thereby producing lamellar rings in some NMO cases. If tissue destruction becomes severe and persistent, demyelinated bands may give rise to necrotic ones, as reported in the case of concentric lacunar leukoencephalopathy in NMO.[41] Thus, AQP4 loss and astrocytopathy may contribute to the development of lamellar demyelination, irrespective of anti-AQP4 antibody-dependent (anti-AQP4

antibody-positive NMO) or -independent conditions (Baló's disease).

Conclusions and future perspectives

The mechanism of antibody-independent AQP4 loss remains to be elucidated. The effects of plentiful inflammatory factors from T cells and macrophages on AQP4 expression in astrocytes should be further studied in vivo and in vitro. Furthermore, the mechanism of simultaneous development of lamellar inflammation in Baló's disease, which is seen pathologically and is also evident by lamellar and serpentine enhancement of the rings on MRI, remains unknown. Step-by-step outward expansion of Baló's concentric rings, together with induction of stress proteins in the outer, normal-appearing white matter, seems to explain only a fraction of the cases with this condition or a later phase of the disease. Increasing evidence suggests that Th17 cells, but not Th1 cells, are responsible for organ-specific autoimmune diseases such as EAE.[58,59] Th17 cells carrying granzyme B have recently been shown to efficiently disrupt BBB tight junctions and loosen the BBB.[60] Therefore, autoimmune Th17 cells may initiate BBB disruption and inflammation in demyelinating disease. Such cell-mediated inflammatory components could also be an important area for future research into Baló's disease, no matter what the target CNS antigens are. I believe that the discovery of antibody-independent AQP4 astrocytopathy opens an intriguing field of research in Baló's disease, and that Baló's disease could be a possible link between MS and NMO, both of which can develop concentric rings of Baló. This hypothesis is based on descriptive data and it should be tested by future experimental studies.

Acknowledgments

I thank Drs Takeshi Matsuoka, Satoshi O Suzuki, Katsuhisa Masaki, Noriko Isobe, Tomomi Yonekawa and Takuya Matsushita for their help with neuropathological and immunological examinations.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

None declared.

References

1. Marburg O. Die so-genannte 'akute multiple Sklerose' (Encephalomyelitis periaxialis scleroticans). *Jahrb Psychiatr* 1906; 28: 213–312.
2. Baló J. Encephalitis periaxialis concentrica. *Arch Neurol Psychiatry* 1928; 19: 242–264.
3. Matsuoka T, Suzuki SO, Iwaki T, Tabira T, Ordinario AT and Kira JI. Aquaporin-4 astrocytopathy in Balo's disease. *Acta Neuropathol* 2010; 120: 651–660.
4. Kuroiwa Y. Concentric sclerosis. In: Koetsier JC (ed.) *Handbook of clinical neurology. Demyelinating disease.* Amsterdam: Elsevier, 1985, pp.409–417.
5. Courville CB. Concentric sclerosis. In: Vinken PJ, Bruyn GW (eds) *Handbook of clinical neurology. Multiple sclerosis and other demyelinating diseases.* Amsterdam: Elsevier, 1970, pp.437–451.
6. Bolay H, Karabudak R, Tacal T, Önol B, Selekler K and Saribas SO. Baló's concentric sclerosis. Report of two patients with magnetic resonance imaging follow-up. *J Neuroimaging* 1996; 6: 98–103.
7. Moore GR, Berry K, Oger JF, Prout AE, Graeb DA and Nugent RA. Baló's concentric sclerosis: surviving normal myelin in a patient with a relapsing-remitting clinical course. *Mult Scler* 2001; 7: 375–382.
8. Karaarslan E, Altintas A, Senol U, et al. Baló's concentric sclerosis: clinical and radiologic features of five cases. *AJNR Am J Neuroradiol* 2001; 22: 1362–1367.
9. Anschel DJ. Reply to the paper by Wiendl et al.: diffusion abnormality in Balo's concentric sclerosis: clues for the pathogenesis. *Eur Neurol* 2006; 55: 111–112.
10. Lindquist S, Bodammer N, Kaufmann J, et al. Histopathology and serial, multimodal magnetic resonance imaging in a multiple sclerosis variant. *Mult Scler* 2007; 13: 471–482.
11. Wang C, Zhang KN, Wu XM, et al. Baló's disease showing benign clinical course and co-existence with multiple sclerosis-like lesions in Chinese. *Mult Scler* 2008; 14: 418–424.
12. Iannucci G, Mascalchi M, Salvi F and Filippi M. Vanishing Balò-like lesions in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2000; 69: 399–400.
13. Adams RD and Kubik CS. The morbid anatomy of the demyelinative diseases. *Am J Med* 1952; 12: 510–546.
14. Makifuchi T. Pathology of concentric sclerosis of Baló's disease. *Neurol Med* 2002; 56: 498–501. (Japanese).
15. Moore GRW, Neumann PE, Suzuki K, Lijtmaer HN, Traugott U and Raine CS. Balo's concentric sclerosis: new observations on lesion development. *Ann Neurol* 1985; 17: 604–611.
16. Kreft KL, Mellema SJ and Hintzen RQ. Spinal cord involvement in Balo's concentric sclerosis. *J Neurol Sci* 2009; 279: 114–117.
17. Yao DL, Webster Hde F, Hudson LD, et al. Concentric sclerosis (Balo): morphometric and in situ hybridization study of lesions in six patients. *Ann Neurol* 1994; 35: 18–30.
18. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M and Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000; 47: 707–717.
19. Lassmann H. Hypoxia-like tissue injury as a component of multiple sclerosis lesions. *J Neurol Sci* 2003; 206: 187–191.
20. Stadelmann C, Ludwin S, Tabira T, et al. Tissue preconditioning may explain concentric lesions in Baló's type of multiple sclerosis. *Brain* 2005; 128: 979–987.

21. Kishimoto R, Yabe I, Niino M, et al. Baló's concentric sclerosis-like lesion in the brainstem of a multiple sclerosis patient. *J Neurol* 2008; 255: 760–761.
22. Graber JJ, Kister I, Geyer H, Khaund M and Herbert J. Neuromyelitis optica and concentric rings of Baló in the brainstem. *Arch Neurol* 2009; 66: 274–275.
23. Chen CJ, Chu NS, Lu CS and Sung CY. Serial magnetic resonance imaging in patients with Balò's concentric sclerosis: natural history of lesion development. *Ann Neurol* 1999; 46: 651–656.
24. Kastrup O, Stude P and Limmroth V. Balo's concentric sclerosis. Evolution of active demyelination demonstrated by serial contrast-enhanced MRI. *J Neurol* 2002; 249: 811–814.
25. Wiendl H, Weissert R, Herrlinger U, Krapf H and Küker W. Diffusion abnormality in Balo's concentric sclerosis: clues for the pathogenesis. *Eur Neurol* 2005; 53: 42–44.
26. Kavanagh EC, Heran MKS, Fenton DM, Lapointe JS, Nugent RA and Graeb DA. Diffusion-weighted imaging findings in Balo concentric sclerosis. *Br J Radiol* 2006; 79: e28–e31.
27. Schaefer PW. Applications of DWI in clinical neurology. *J Neurol Sci* 2001; 186(Suppl 1): S25–S35.
28. Mader I, Herrlinger U, Klose U, Schmidt F and Küker W. Progressive multifocal leukoencephalopathy: analysis of lesion development with diffusion-weighted MRI. *Neuroradiology* 2003; 45: 717–721.
29. Küker W, Ruff J, Gaertner S, Mehnert F, Mäder I and Nagele T. Modern MRI tools for the characterization of acute demyelinating lesions: value of chemical shift and diffusion-weighted imaging. *Neuroradiology* 2004; 46: 421–426.
30. Muir KW, Buchan A, von Kummer R, Rother J and Baron JC. Imaging of acute stroke. *Lancet Neurol* 2006; 5: 755–768.
31. Balasubramanya KS, Kovoor JME, Jayakumar PN, et al. Diffusion-weighted imaging and proton MR spectroscopy in the characterization of acute disseminated encephalomyelitis. *Neuroradiology* 2007; 49: 177–183.
32. Castriota-Scanderbeg A, Sabatini U, Fasano F, et al. Diffusion of water in large demyelinating lesions: a follow-up study. *Neuroradiology* 2002; 44: 764–767.
33. Rovira A, Pericot I, Alonso J, Rio J, Grivé E and Montalban X. Serial diffusion-weighted MR imaging and proton MR spectroscopy of acute large demyelinating brain lesions: case report. *AJNR Am J Neuroradiol* 2002; 23: 989–994.
34. Rosso C, Remy P, Creange A, Brugieres P, Cesaro P and Hosseini H. Diffusion-weighted MR imaging characteristics of an acute strokelike form of multiple sclerosis. *AJNR Am J Neuroradiol* 2006; 27: 1006–1008.
35. Balashov KE, Aung LL, Dhib-Jalbut S, Keller IA. Acute multiple sclerosis lesion: conversion of restricted diffusion due to vasogenic edema. *J Neuroimaging* 2009 (accessed 28 January 2011).
36. Pichiecchio A, Tavazzi E, Maccabelli G, et al. What insights have new imaging techniques given into aggressive forms of MS -different forms of MS or different from MS? *Mult Scler* 2009; 15: 285–293.
37. Przeklasa-Auth M, Ovbiagele B, Yim C and Shewmon DA. Multiple sclerosis with initial stroke-like clinicoradiologic features: case report and literature review. *J Child Neurol* 2010; 25: 732–737.
38. Malhotra HS, Jain KK, Agarwal A, et al. Characterization of tumefactive demyelinating lesions using MR imaging and in-vivo proton MR spectroscopy. *Mult Scler* 2009; 15: 193–203.
39. Chen CJ. Serial proton magnetic resonance spectroscopy in lesions of Balo concentric sclerosis. *J Comput Assist Tomogr* 2001; 25: 713–718.
40. Field EJ, Miller H and Russell DS. Observations on glial inclusion bodies in a case of acute disseminated sclerosis. *J Clin Pathol* 1962; 15: 278–284.
41. Currie S, Roberts AH and Urich H. The nosological position of concentric lacunar leucoencephalopathy. *J Neurol Neurosurg Psychiatry* 1970; 33: 131–137.
42. Itoyama Y, Tateishi J and Kuroiwa Y. Atypical multiple sclerosis with concentric or lamellar demyelinated lesions: two Japanese patients studied post mortem. *Ann Neurol* 1985; 17: 481–487.
43. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; 364: 2106–2112.
44. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS and Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005; 202: 473–477.
45. Misu T, Fujihara K, Kakita A, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain* 2007; 130: 1224–1234.
46. Roemer SF, Parisi JE, Lennon VA, et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain* 2007; 130: 1194–1205.
47. Masaki K, Matsuoka T, Suzuki SO, et al. Extensive aquaporin-4 (AQP4) loss in Balo's concentric sclerosis in the absence of anti-AQP4 antibody. (Abstract) *Mult Scler* 2010 (in press).
48. Kinoshita M, Nakatsuji Y, Kimura T, et al. Neuromyelitis optica: passive transfer to rats by human immunoglobulin. *Biochem Biophys Res Commun* 2009; 386: 623–627.
49. Bennett JL, Lam C, Kalluri SR, et al. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol* 2009; 66: 617–629.
50. Bradl M, Misu T, Takahashi T, et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. *Ann Neurol* 2009; 66: 630–643.
51. Frydenlund DS, Bhardwaj A, Otsuka T, et al. Temporary loss of perivascular aquaporin-4 in neocortex after transient middle cerebral artery occlusion in mice. *Proc Natl Acad Sci U S A* 2006; 103: 13532–13536.
52. Fujita Y, Yamamoto N, Sobue K, et al. Effect of mild hypothermia on the expression of aquaporin family in cultured rat astrocytes under hypoxic condition. *Neurosci Res* 2003; 47: 437–444.
53. Lee M, Lee SJ, Choi HJ, et al. Regulation of AQP4 protein expression in rat brain astrocytes: role of P2X7 receptor activation. *Brain Res* 2008; 1195: 1–11.

54. Meng S, Qiao M, Lin L, Del Bigio MR, Tomanek B and Tuor UI. Correspondence of AQP4 expression and hypoxic-ischaemic brain oedema monitored by magnetic resonance imaging in the immature and juvenile rat. *Eur J Neurosci* 2004; 19: 2261–2269.
55. Papadopoulos MC, Manley GT, Krishna S and Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J* 2004; 18: 1291–1293.
56. 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.
57. Li L, Zhang H and Verkman AS. Greatly attenuated experimental autoimmune encephalomyelitis in aquaporin-4 knockout mice. *BMC Neurosci* 2009; 10: 94.
58. Cua DJ, Sherlock J, Chen Y, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003; 421: 744–748.
59. Weaver CT, Hatton RD, Mangan PR and Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; 25: 821–852.
60. Kebir H, Kreymborg K, Ifergan I, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 2007; 13: 1173–1175.

MULTIPLE SCLEROSIS JOURNAL | MSJ

Multiple Sclerosis Journal
17(7) 885–887

DOI: 10.1177/1352458510391690
msj.sagepub.com
SAGE

Wall-eyed bilateral internuclear ophthalmoplegia (WEBINO) syndrome in a patient with neuromyelitis optica spectrum disorder and anti-aquaporin-4 antibody

Koji Shinoda[1], Takuya Matsushita[2], Konosuke Furuta[1], Noriko Isobe[1], Tomomi Yonekawa[1], Yasumasa Ohyagi[1] and Jun-ichi Kira[1]

Abstract

This report describes, for the first time, an occurrence of wall-eyed bilateral internuclear ophthalmoplegia (WEBINO) in a 19-year-old female with neuromyelitis optica (NMO) spectrum disorder, who had anti-aquaporin-4 (AQP4) antibody. A high signal intensity lesion on T2-weighted MRI was detected in the midbrain tegmentum adjacent to the aqueduct, and presumably involved the medial longitudinal fasciculus bilaterally at the caudal levels. Plasma exchange resolved both WEBINO syndrome and the midbrain lesion. Although WEBINO syndrome is occasionally reported in multiple sclerosis patients, diagnosis of NMO should not be excluded in patients with WEBINO syndrome, because AQP4 is expressed abundantly around the periaqueductal region.

Keywords

aquaporin-4, MRI, neuromyelitis optica, WEBINO syndrome

Date received: 8th September 2010; revised: 20th October 2010; accepted: 20th October 2010

Introduction

Wall-eyed bilateral internuclear ophthalmoplegia (WEBINO) is a rarely reported syndrome. Clinically, patients with WEBINO syndrome demonstrate exotropia in the primary eye position and a bilateral adduction deficit with abducting nystagmus on horizontal gaze.[1] The causal lesions are thought to be bilateral medial longitudinal fasciculi (MLF). WEBINO syndrome has been reported in patients with cerebrovascular diseases, multiple sclerosis (MS), brainstem tumor, and supranuclear progressive palsy.[1–3] Here, for the first time, we report a patient with neuromyelitis optica (NMO) spectrum disorder (NMOSD) who had a specific anti-aquaporin-4 (AQP4) antibody[4,5] and presented with WEBINO syndrome.

Case report

A 19-year-old female was admitted to our hospital with a chief complaint of double vision for 2 days. Eight months before admission, she had been referred and admitted to our hospital because of an acute onset of double vision, dysphagia, dysarthria and right-sided weakness and paresthesia. At the first admission, her anti-nuclear antibody (ANA) and anti-SS-A antibody were positive. Anti-AQP4 antibody was also positive using an immunofluorescence assay.[6] The CSF was hypercellular (38/μl, all mononuclear cells) with slightly elevated levels of total protein (52 mg/dl) and myelin basic protein (815 pg/ml), but no oligoclonal bands were found. Her brain and spinal cord MRI revealed

[1]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Japan.
[2]Department of Clinical Neuroimmunology, Graduate School of Medical Sciences, Kyushu University, Japan.

Corresponding author:
Jun-ichi Kira, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 311 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
Email: kira@neuro.med.kyushu-u.ac.jp

hyperintense areas on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images in the hypothalamus bilaterally and from the medulla to C1 in the spinal cord without contrast enhancement (Figure 1A and B). In the spinal cord, T2-weighted images revealed high signal intensity lesions at the levels C4–7 (Figure 1A), T9–10 and T12–L1. We diagnosed her as having an NMOSD due to the presence of anti-AQP4 antibody, longitudinally extensive spinal cord lesions (LESCLs) and atypical brain lesions, which collectively are considered to be characteristic of NMO. Intravenous methylprednisolone pulse therapy (1000 mg daily for 3 days) followed by oral prednisolone (50 mg/day with gradual taper) was initiated. On discharge, she had only slight hemiparesis and hypesthesia on the right side of her body and slight ataxia of all four limbs.

At the second admission, neurological examination revealed normal convergence, a sluggish light reflex, adduction deficit and exotropia of the right eye, monocular abducting nystagmus of the left eye on leftward gaze, right-side deviation on tongue protrusion and Lhermitte's sign. The CSF had minimal hypercellularity (6/μl). ANA, anti-SS-A antibody and anti-AQP4 antibody were again all positive in her serum. Methylprednisolone pulse therapy (1000 mg daily for 3 days) was administered, but there was no effect on her double vision. Additionally, an adduction deficit of the left eye and exotropia of either eye on primary

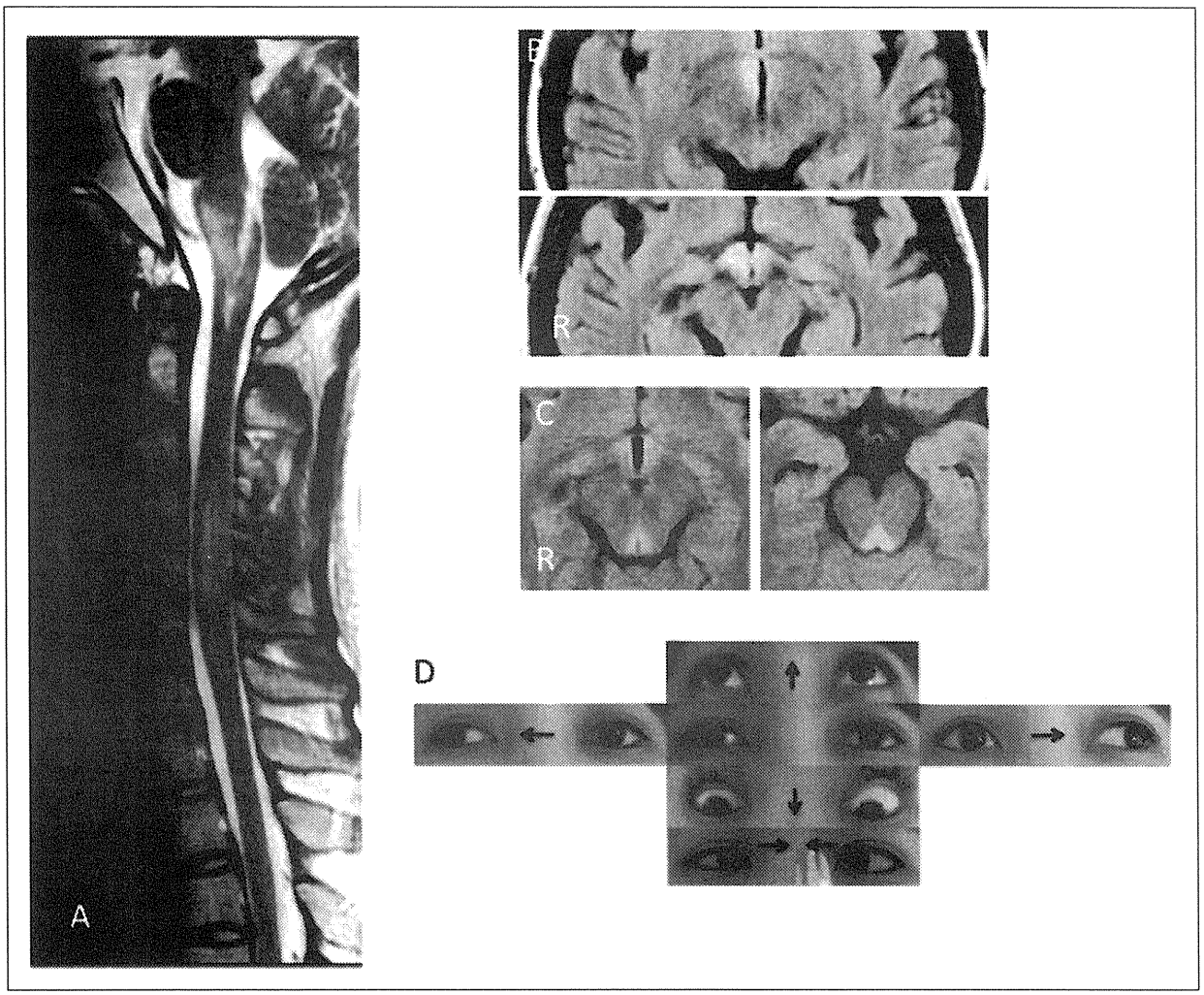

Figure 1. T2-weighted image (TR = 2500 ms, TE = 108 ms) at the first admission demonstrates a continuous hyperintense lesion from the medulla to C1, and a longitudinally extensive spinal cord lesion from C4 to C7 (**A**). FLAIR images (TR = 9000 ms, TE = 97 ms) show band-like hyperintense lesions in the hypothalamus bilaterally (B). MRI at the time of appearance of WEBINO syndrome shows a high signal intensity lesion in the midbrain tegmentum adjacent to the aqueduct on FLAIR images (TR = 9000 ms, TE = 110 ms) (**C**). Photographs of ocular movement demonstrating exotropia of either eye in the primary eye position and an adduction deficit of either eye on attempted lateral gaze (**D**). Convergence is intact. The arrows indicate the directions of attempted gaze.
FLAIR: fluid-attenuated inversion recovery, WEBINO: wall-eyed bilateral internuclear ophthalmoplegia.

gaze position occurred. At this point, she had bilateral adduction deficit with dissociated abducting nystagmus of abducting eyes on lateral gaze and exotropia of either eye (Figure 1D), which met the criteria for WEBINO syndrome.[1] Head MRI disclosed a new hyperintense lesion on T2-weighted and FLAIR images in the dorsal portion of the midbrain around the aqueduct (Figure 1C). Following corticosteroid therapy, plasma exchange was performed three times, with marked improvement of her double vision and disappearance of her exotropia.

Discussion

We report the first case of a patient with anti-AQP4 antibody displaying WEBINO syndrome. NMO consists of optic neuritis and myelitis, and is usually associated with LESCLs extending over three or more vertebral segements.[7] Initially, NMO was considered not to be accompanied by brain lesions; however, the discovery of NMO-IgG revealed that atypical brain lesions, such as bilateral diencephalic lesions, large confluent cerebral hemispheric lesions, and medullary lesions extending from the cervical cord, were not rarely encountered in this condition.[8] Although the present patient had no optic neuritis, the presence of anti-AQP4 antibody, LESCLs and atypical brain lesions, namely bilateral hypothalamic lesions and a medullary lesion extending from the C1 level of the spinal cord, strongly suggests that she was suffering from the early stage of an NMOSD.[9]

WEBINO syndrome is considered to be caused by lesions involving the bilateral MLF and medial rectus subnucleus.[1] However, it is assumed that an involvement of the oculomotor nucleus is not essential for exotropia, and that an imbalance between bilateral paramedian pontine reticular formation (PPRF) activities on eye fixation is responsible.[10] In our patient, the lesion responsible for WEBINO syndrome, as determined by MRI, is the midbrain tegmentum lesion adjacent to the aqueduct. This lesion is likely to involve the MLF near the oculomotor nucleus bilaterally.

WEBINO syndrome has repeatedly been reported in patients with MS, in which brainstem lesions involving bilateral MLF frequently develop. In patients with NMO, the periaqueductal region, where an expression of aquaporin-4 is supposed to be high,[9] is occasionally involved.[6] The observed efficacy of plasma exchange for treating WEBINO syndrome suggests that humoral factors, such as anti-AQP4 antibody, contributed to its development in this patient. Hence, because not only MS but also anti-AQP4 antibody-positive NMO patients can present with WEBINO syndrome, diagnosis of NMO should not be excluded in patients with WEBINO syndrome, or even bilateral MLF syndrome.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors report no conflicts of interest.

References

1. McGettrick P and Eustace P. The w.e.h.i.n.o. syndrome. *Neuro-ophthalmol* 1985; 5: 109–115.
2. Strominger MB, Mincy EJ, Strominger AI and Strominger NL. Bilateral internuclear ophthalmoplegia with absence of convergent eye movements. Clinicopathologic correlation. *J Clin Neuroophthalmol* 1986; 6: 57–65.
3. Ushio M, Iwasaki S, Chihara Y and Murofushi T. Wall-eyed bilateral internuclear ophthalmoplegia in a patient with progressive supranuclear palsy. *J Neuroophthalmol* 2008; 28: 93–96.
4. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; 364: 2106–2112.
5. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS and Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005; 202: 473–477.
6. Matsushita T, Isobe N, Matsuoka T, et al. Aquaporin-4 autoimmune syndrome and anti-aquaporin-4 antibody-negative opticospinal multiple sclerosis in Japanese. *Mult Scler* 2009; 15: 834–847.
7. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF and Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; 66: 1485–1489.
8. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF and Weinshenker BG. Brain abnormalities in neuromyelitis optica. *Arch Neurol* 2006; 63: 390–396.
9. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ and Weinshenker BG. The spectrum of neuromyelitis optica. *Lancet Neurol* 2007; 6: 805–815.
10. Chen C-M and Lin S-H. Wall-eyed bilateral internuclear ophthalmoplegia from lesions at different levels in the brainstem. *J Neuroophthalmol* 2007; 27: 9–15.

Brain Pathology ISSN 1015-6305

RESEARCH ARTICLE

Reappraisal of Aquaporin-4 Astrocytopathy in Asian Neuromyelitis Optica and Multiple Sclerosis Patients

Takeshi Matsuoka[1,2]; Satoshi O. Suzuki[2]; Toshihiko Suenaga[3]; Toru Iwaki[2]; Jun-ichi Kira[1]

[1] Departments of Neurology and [2] Neuropathology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
[3] Department of Neurology, Tenri Hospital, Tenri, Japan.

Keywords
antibody, aquaporin-4, astrocyte, multiple sclerosis, neuromyelitis optica.

Corresponding author:
Satoshi O. Suzuki, MD, PhD, Department of Neuropathology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (E-mail: *sosuzuki@np.med.kyushu-u.ac.jp*)

Received 11 August 2010; accepted 9 December 2010.

doi:10.1111/j.1750-3639.2011.00475.x

Abstract

Selective aquaporin-4 (AQP4) loss and vasculocentric complement and immunoglobulin deposition are characteristic of neuromyelitis optica (NMO). We recently reported extensive AQP4 loss in demyelinated and myelinated layers of Baló's lesions without perivascular immunoglobulin and complement deposition. We aimed to reappraise AQP4 expression patterns in NMO and multiple sclerosis (MS). We evaluated AQP4 expression relative to glial fibrillary acidic protein, extent of demyelination, lesion staging (CD68 staining for macrophages), and perivascular deposition of complement and immunoglobulin in 11 cases with NMO and NMO spectrum disorders (NMOSD), five with MS and 30 with other neurological diseases. The lesions were classified as actively demyelinating (n = 66), chronic active (n = 86), chronic inactive (n = 48) and unclassified (n = 12). Six NMO/NMOSD and two MS cases showed preferential AQP4 loss beyond the demyelinated areas, irrespective of lesion staging. Five NMO and three MS cases showed AQP4 preservation even in actively demyelinating lesions, despite grave tissue destruction. Vasculocentric deposition of complement and immunoglobulin was detected only in NMO/NMOSD patients, with less than 30% of actively demyelinating lesions showing AQP4 loss. Our present and previous findings suggest that antibody-independent AQP4 loss can occur in heterogeneous demyelinating conditions, including NMO, Baló's disease and MS.

Abbreviations: ALS = amyotrophic lateral sclerosis; AQP4 = aquaporin-4; BBB = blood-brain barrier; BCS = Baló's concentric sclerosis; CNS = central nervous system; GFAP = glial fibrillary acidic protein; H & E = hematoxylin and eosin, IgG = immunoglobulin G; KB = Klüver-Barrera; LESCL = longitudinally extensive spinal cord lesion; MG = myasthenia gravis; MS = multiple sclerosis; MRI = magnetic resonance imaging; NMO = neuromyelitis optica; NMOSD = neuromyelitis optica spectrum disorder; OSMS = opticospinal multiple sclerosis; SCD = spinocerebellar degeneration; SPG = spastic paraplegia.

INTRODUCTION

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are inflammatory demyelinating diseases of the central nervous system (CNS). The pathological hallmark in MS is sharply demarcated demyelinating plaques with axons relatively preserved, suggesting autoimmune attacks targeting CNS myelin. By contrast, NMO shows selective and severe attacks on both axons and myelin of the optic nerves and spinal cord, resulting in necrotic cavitation. In this condition, longitudinally extensive spinal cord lesions (LESCLs) extending over three vertebral segments are characteristic on magnetic resonance imaging (MRI) (54).

Although the nosological position of NMO has long been a matter of debate, the recent discovery of a specific IgG against NMO, designated NMO-IgG (28), suggests that NMO is distinct from MS and has a fundamentally different etiology. This IgG targets the aquaporin-4 (AQP4) water channel protein (29), which is strongly expressed on astrocyte foot processes at the blood–brain barrier (BBB) (18). Autopsied NMO cases show a loss of AQP4 immunostaining in inflammatory lesions (38, 45). The vasculocentric deposition of complement and immunoglobulins in NMO lesions (30) suggests a humoral immune attack against AQP4 on astrocytes, especially as the NMO-IgG/anti-AQP4 antibody is cytotoxic to astrocytes *in vitro* and *in vivo* in the presence of complement (3, 5, 19–21, 46, 47, 52).

However, the predictive value of the NMO-IgG/anti-AQP4 antibody is only moderate; 30%–73% in Caucasians (9, 10, 17, 28, 42), 63% in northern Japanese with opticospinal MS (OSMS) (39), 27% in southern Japanese with OSMS (31) and 33% in Caribbean people (6). On the other hand, 5%–15% of MS cases are positive

for NMO-IgG or anti-AQP4 antibody. Pittock *et al* (43) described that 10% of NMO-IgG-positive patients had brain lesions that were indistinguishable from those in MS patients, whereas some patients also showed extensive brain lesions (34, 36, 43). By contrast, LESCLs are seen in about a quarter of classical Asian MS patients (8, 32, 37, 49), compared with 12.5% of Caucasian MS patients (4). Asian patients with MS show necrotizing lesions with occasional cavity formation in the spinal cord, optic nerve and cerebrum (12, 14, 40, 50), reflecting severe inflammation. These findings suggest considerable overlap between NMO and MS, especially in Asians.

In addition, Baló's concentric sclerosis (BCS), a rare variant of MS with huge brain lesions showing concentric rings of alternating demyelination and normal myelin layers, is more common in Asians, such as Filipinos (25), southern Han Chinese (53) and Taiwanese (7), than in other races. Asian MS patients can also show concentric demyelinating lesions in the spinal cord and the optic chiasm pathologically (16), representing an intermediate between BCS and MS. We recently reported extensive AQP4 loss in both demyelinated and myelinated layers of BCS lesions in the absence of perivascular immunoglobulin and complement deposition (33). Interestingly, development of BCS-like lesions in the brainstem on MRI was recently reported in Asian patients with not only classical MS (23) but also NMO (11). Other than two pivotal neuropathological studies (38, 45), only a few case reports have examined AQP4 expression in NMOs with varying results (13, 24, 51, 57). Furthermore, although AQP4 expression in MS has also been examined in some studies (38, 45, 48), there are inconsistencies among their conclusions. We therefore decided to perform a systematic immunohistological reappraisal of AQP4 expression relative to astrocyte marker expression, the extent of demyelination, lesion staging and the perivascular deposition of complement and immunoglobulin in Asian NMO and MS patients to clarify the contribution of astrocyte damage, including AQP4 loss to the lesion formation, in these conditions.

MATERIALS AND METHODS

Autopsy tissue and patient characterization

The study was performed on archival autopsied brain, optic nerve and spinal cord materials from 10 NMO cases, including one anti-AQP4 antibody-positive case, one case with NMO spectrum disorder (NMOSD), and five cases with MS. All but the anti-AQP4 antibody-positive case from Tenri Hospital were from the Department of Neuropathology, Kyushu University. NMO/NMOSD diagnosis was based on the Wingerchuk criteria (54–56), while MS was diagnosed according to the Poser criteria (44). The clinical findings are summarized in Table 1. The median age at autopsy was 44.0 years old (range 28–88) in the NMO/NMOSD cases (nine females and two males) and 39.0 years old (range 12–52) in the MS cases (three females and two males). Disease durations ranged from 0.4 to 17.0 years in the NMO/NMOSD group (median, 4.7 years), and from 0.7 to 21.0 years in the MS group (median, 3.1 years). In addition, we used the same set of control cases with other neurological diseases as in our previous study (33): myasthenia gravis (MG) (n = 2), spastic paraplegia type 2 (n = 1), amyotrophic lateral sclerosis (n = 6), hippocampal sclerosis with temporal lobe epilepsy (n = 5), muscular dystrophy (n = 1), encephalitis (n = 3), including one with anti-N-methyl-D-aspartate receptor antibody and another with anti-thyroglobulin antibody, spinocerebellar degeneration (n = 1),

Table 1. Summary of clinical and pathological findings of Japanese patients with neuromyelitis optica and multiple sclerosis. Abbreviations: MS = multiple sclerosis; N.A. = not applicable; NMO = neuromyelitis optica (Wingerchuk *et al* (55, 56)); NMOSD = neuromyelitis optica spectrum disorders (Wingerchuk *et al* (57)).

Autopsy	Age (years)	Sex	Disease duration (years)	Relapse rate	Clinically estimated sites of lesions	Pathologically determined sites of lesions
NMO-1	44	F	3.8	1.6	O2, S6	O, S, Bs, Cr, Cl
NMO-2	44	F	1.8	2.8	O2, Bs3, S2	O, S, Bs, Cr
NMO-3	48	F	0.5	4.0	O2, Bs1, S1	O, S, Bs, Cr
NMO-4	32	M	6.3	1.1	O1, Bs2, S7	O, S, Bs, Cl, Cr
NMO-5	28	F	4.7	0.6	O3, Bs2, S2	O, S, Bs, Cl, Cr
NMO-6	35	F	7.0	1.4	O4, Bs4, S4	O, S, Bs, Cr
NMO-7	37	F	10.8	1.5	O9, Bs2, S14	O, S, Bs, Cl, Cr
NMO-8	47	F	8.3	0.1	O2, Bs1, S2	O, S, Bs, Cr
NMO-9	54	F	4.0	1.3	O1, S6	O, S
NMO-10*	37	F	17.0	1.1	O8, Bs1, S9, Cr3	Bs, S, Cr
NMOSD	88	M	0.4	0.0	S1	O, S
MS-1	12	F	5.0	2.2	O6, Bs1, S2, Cr3	O, Bs, S, Cr, Cl
MS-2	35	M	3.1	1.0	O1, Bs2, S2, Cr2	O, Bs, S, Cr
MS-3	52	F	1.3	1.5	Bs1, Cr3	Bs, Cr
MS-4	45	F	0.7	1.4	Bs2, S2	O, S, Bs
MS-5	39	M	21.0	0.5	O1, Bsx, Clx, Crx	O, Bs, Cl, Cr

Lesion sites in clinical exacerbations: O = optic nerve; S = spinal cord; Bs = brainstem; Cl = cerebellum; Cr = cerebrum. Numbers indicate exacerbations in each lesion site (eg, O2 represents two episodes of optic neuritis). Asterisk indicates a NMO case with known anti-AQP4 antibody seropositivity.

Table 2. Antibodies used for immunohistochemistry. Abbreviations: AQP4 = aquaporin-4, GFAP = glial fibrillary acidic protein; N.D. = not done.

Antibody	Type	Dilution	Antigen retrieval	Source
Aquaporin-4				
AQP4	rabbit polyclonal	1:500	N.D	Santa Cruz Biotechnology, California, USA
Complement				
C3d	rabbit polyclonal	1:1000	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark
C9neo	mouse monoclonal	1:1000	Autoclave/10 mM citrate buffer	Abcam plc, Cambridge, UK
Macrophage/microglia				
CD68	mouse monoclonal	1:200	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark
Astrocyte				
GFAP	rabbit polyclonal	1:1000	N.D.	DakoCytomation, Glostrup, Denmark
Immunoglobulin				
IgG	rabbit polyclonal	1:10 000	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark
IgM	rabbit polyclonal	1:10 000	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark
Lymphocyte				
CD45RO	mouse monoclonal	1:200	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark
CD20	mouse monoclonal	1:200	Autoclave/10 mM citrate buffer	DakoCytomation, Glostrup, Denmark

vasculitis (n = 3), cerebral infarction (n = 1), Pick's disease (n = 1), progressive supranuclear palsy (n = 3) and multiple system atrophy (n = 3).

Tissue preparation and immunohistochemistry

Autopsied specimens were fixed in 10% formalin and processed into paraffin sections. Sections were routinely stained with hematoxylin and eosin (H & E), Klüver-Barrera (KB), and Bodian or Bielschowsky silver impregnation. Primary antibodies for immunohistochemistry are listed in Table 2. All sections were deparaffinized in xylene and rehydrated in an ethanol gradient. Endogenous peroxidase activity was blocked with 0.3% H_2O_2/methanol. The sections were then incubated with primary antibody at 4°C overnight. After rinsing, the sections were subjected to either a streptavidin-biotin complex method or an enhanced indirect immunoperoxidase method using Envision (DakoCytomation, Glostrup, Denmark). Immunoreactivity was detected using 3,3′-diaminobenzidine and sections were counterstained with hematoxylin. Immunohistochemistry for activated complement, immunoglobulins, T cell and B cell markers was performed in randomly selected lesions.

Staging of demyelinating lesions

We classified demyelinating lesions into the following three stages: actively demyelinating lesions, chronic active lesions and chronic inactive lesions based on the density of infiltrating macrophages (26). Briefly, actively demyelinating lesions had active destructive lesions densely and diffusely infiltrated with macrophages phagocytosing myelin debris, as identified by Luxol fast blue staining. Chronic active lesions were those showing hypercellularity of macrophages restricted to the periphery of the lesions. Chronic inactive lesions were those showing no increase in macrophages throughout the lesions.

According to the staging protocol, NMO lesions (n = 149) were classified as 42 actively demyelinating lesions, 72 chronic active lesions and 35 chronic inactive lesions, whereas MS lesions (n = 51) were classified into 24 actively demyelinating lesions, 14 chronic active lesions and 13 chronic inactive lesions. In addition, we noted 12 lesions in NMO (seven lesions) and MS cases (five lesions) that did not meet the staging criteria. All of these lesions had perivascular deposition of both activated complement and immunoglobulins, but no macrophage infiltration, and lacked any myelin or AQP4 loss. These lesions were thus analyzed separately.

Figure 1. Loss of AQP4 expression in active lesions of NMO. (**A–F**) Serial sections of an actively demyelinating cerebral lesion from NMO-10 with known anti-AQP4 antibody seropositivity representing Pattern A. Arrowheads indicate the same blood vessel. **A.** Sharply demarcated demyelinating plaque is noted in the cerebral white matter. **B.** GFAP immunopositivity is decreased in the lesion. **C.** AQP4 immunopositivity is lost beyond the area with myelin/GFAP loss. **D.** Numerous macrophages contain myelin debris (arrowheads). **E.** Degeneration of vascular foot processes is evident by GFAP immunostaining. **F.** AQP4 immunoreactivity is absent even in the degenerated astrocytes. (**G–O**) Serial sections of corpus callosum lesions from NMO-4 representing Patterns A & N. **G.** The corpus callosum demonstrates severe demyelination with tissue necrosis (dagger). **H.** CD68-positive macrophages diffusely infiltrate the lesions. **I.** GFAP immunostaining shows numerous reactive astrocytes in the surrounding region, but it decreases in the necrotic lesion center and focal perivascular areas with positive myelin staining (arrowhead in G and I). **J.** AQP4 immunostaining reveals a pattern similar to that of GFAP staining, but decreases more extensively in the still-myelinated area indicated by the asterisk in G. **K.** High magnification of the necrotic area indicated by the dagger in G. Numerous foamy macrophages containing myelin debris are present. (L–O) High magnification of the still-myelinated area indicated by the asterisk in G. **L.** Still-preserved myelin with some foamy macrophages without myelin debris. **M.** CD68-immunopositive foamy macrophages are present among the preserved myelin. **N.** GFAP immunostaining reveals degenerated astrocytes and astrocytic vascular foot processes. **O.** AQP4 immunoreactivity is completely lost despite the presence of astrocytes. **A, D, G, K, L, KB; B, E, I, N**, GFAP immunohistochemistry (IHC); **C, F, J, O**, AQP4 IHC; H, M, CD68 IHC. Scale bar = 1 mm (**A–C**); 300 μm (**G–J**); 50 μm (**E, F, L–O**); 20 μm (**D, K**). AQP4 = aquaporin-4; GFAP = glial fibrillary acidic protein; KB = Klüver-Barrera staining; NMO = neuromyelitis optica.

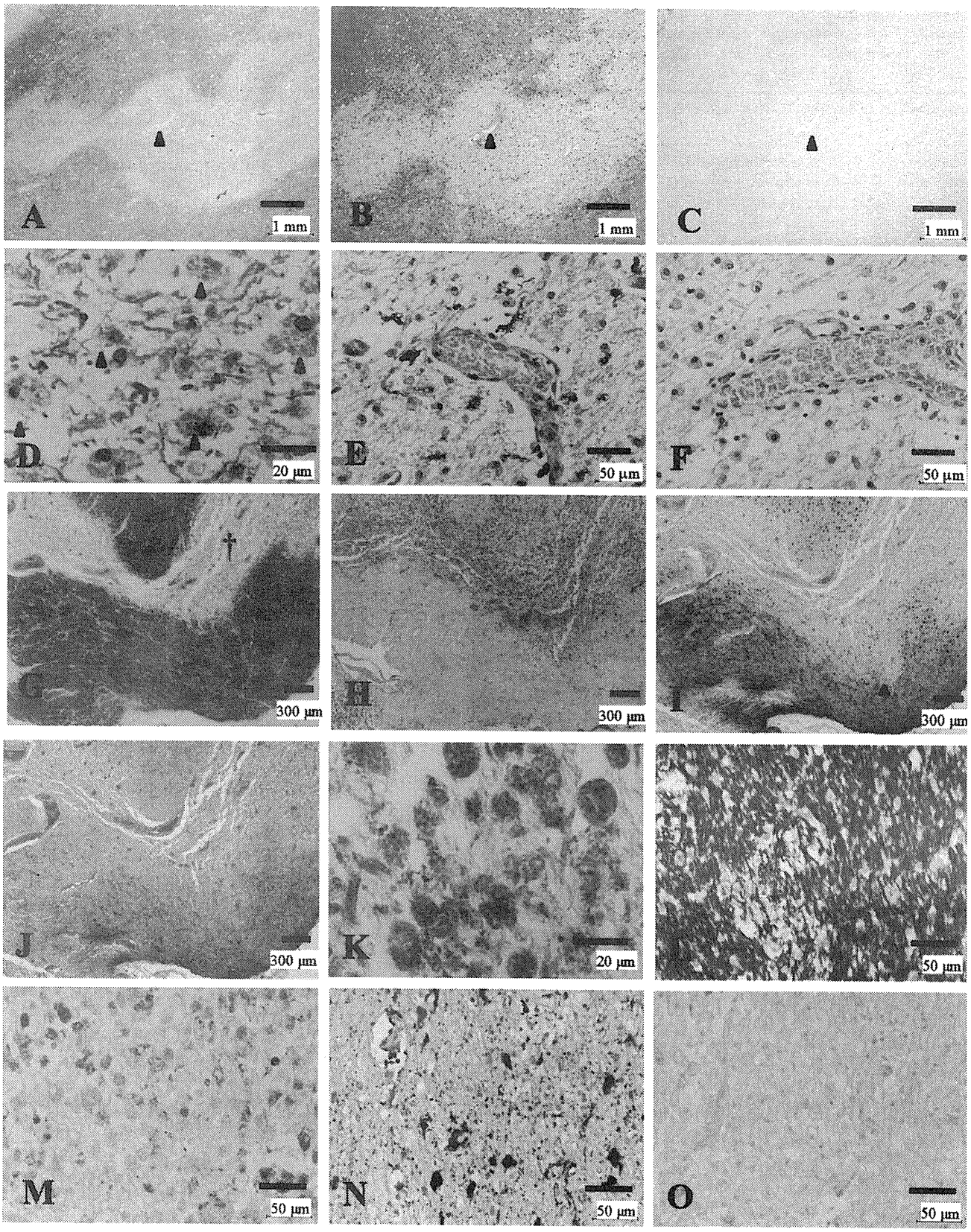

Table 3. Classification of demyelinating lesions according to aquaporin-4 and glial fibrillary acid protein immunostaining and Klüver-Barrera staining. Abbreviations: AQP4 = aquaporin-4; GFAP = glial fibrillary acidic protein; KB = Klüver-Barrera staining.

Lesion pattern	Extents of AQP4 and myelin loss	GFAP expression
Pattern A	AQP4 loss or decrease > myelin loss	(+)
Pattern B	AQP4 loss or decrease = myelin loss	(+)
Pattern C	AQP4 loss or decrease < myelin loss	(+)
Pattern D	AQP4 totally preserved in areas of myelin loss	(+)
Pattern N	necrosis or cavity formation	(–)

Comparison of AQP4 expression with myelin loss and astrogliosis

For each lesion, we compared the level of AQP4 expression with the degree of myelin loss and the level of glial fibrillary acidic protein (GFAP) expression. AQP4 expression levels in region-matched unaffected areas (ie, gray matter or white matter) in the same section were used as an internal control. When the presence of astrocytes was not confirmed by GFAP immunostaining caused by total replacement of a lesion with foamy macrophages, or when cavity formation was evident, the lesion was classified as a destructive necrotic lesion. Accordingly, 15 lesions with severe necrosis or cavity formation (nine in NMO patients and six in MS patients) were determined.

Using serial sections of demyelinating lesions, we divided the expression patterns of AQP4 immunoreactivity relative to the intensity of GFAP immunoreactivity and myelin (KB) staining into the following five patterns (Table 3): Pattern A (area of diminished AQP4 immunoreactivity extending over that of myelin loss), Pattern B (area of diminished AQP4 immunoreactivity conforming with that of myelin loss), Pattern C (area of diminished AQP4 immunoreactivity less than that of myelin loss), Pattern D (preserved AQP4 immunoreactivity with loss of myelin staining) and Pattern N (necrosis or cavity formation; GFAP-negative,

Table 4. Summary of aquaporin-4 immunoreactivity patterns in demyelinating lesions in cases with neuromyelitis optica (NMO) or NMO spectrum disorders. Abbreviations: AQP4 = aquaporin-4; NMO = neuromyelitis optica; NMOSD = neuromyelitis optica spectrum disorder; N.A. = specimen not available.

Autopsy	Stage	Cerebrum	Brainstem	Cerebellum	Spinal cord	Optic nerve
Preferential AQP4 loss or decrease						
NMO-2	Active	A (1)	A&N (1)	N.A.	B&N (1)	N.A.
	Chronic active	B&N (1), C&N (1)			A&N (3), B&N (1)	
	Chronic inactive	C (2)			C&N (1)	
NMO-3	Active	B&N (1)				A&N (1)
	Chronic active	B&N (1), C&N (1)	A (1)		A&N (3)	A&N (1)
	Chronic inactive	C&N (3)				
NMO-4	Active	A (1), A&N (3), B (1), B&N (1)	A&N (2), B&N (1)	B (2)	B&N (1)	D (1)
	Chronic active	B&N (1)	B (2)		A&N (1), B&N (1), C&N (1)	
	Chronic inactive	D (1)				
NMO-7	Active		A (3), B (1)		A&N (2)	
	Chronic active	C (3)	A (3), B (1), C (1), C&N (1)			
	Chronic inactive	C (1), D (2)	C&N (2)			D&N (1)
NMO-10	Active	A (1), A&N (1), N (1)				N.A.
	Chronic active	B (2), B&N (1), C&N (1)	C&N (1)		A&N (1), C&N (1)	
	Chronic inactive	D (1), N (1)				
NMOSD	Active				A&N (1), B&N (1), N (1)	
	Chronic active				B&N (1)	
	Chronic inactive					D (1)
Preserved AQP4 expression						
NMO-1	Active	D&N (1)	N (2)			
	Chronic active		D (1)		D (1)	D (1)
	Chronic inactive	D&N (1)				
NMO-5	Active	D&N (1), D (3)				
	Chronic active	D (5), D&N (4)	D&N (1), D (2)	D (2)		
	Chronic inactive	D (3)	D&N (1)		D&N (1)	D (1)
NMO-6	Active	D (1)				
	Chronic active	D (5), D&N (2)	D&N (1)	D (1)		
	Chronic inactive	D (1)	D&N (1)		D (1), D&N (1)	D&N (1)
NMO-8	Chronic active	D (1), D&N (5)	D&N (1)		D&N (1)	D (1)
	Chronic inactive	D (1), D&N (1)	D (2), N (1)			N (1)
NMO-9	Active				D&N (1), N (2)	D (1)
	Chronic active				D&N (1)	

Blank cell = No lesions.