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SHORT COMMUNICATION

Brain immunohistopathological study in a patient with anti-NMDAR encephalitis

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Background and purpose: Anti-*N*-methyl-D-asparate (NMDA) receptor encephalitis is thought to be antibody-mediated. To perform an immunohistopathological study of the inflammatory reaction in a brain biopsy performed before immunomodulatory treatments in a patient with anti-NMDA receptor encephalitis.

Methods: An immunohistochemical study was performed using CD3, CD68, CD20, CD138 and CD1a antibodies.

Results: Prominent B-cell cuffing was present around brain vessels accompanied by some plasma cells, while macrophages and T cells were scattered throughout the brain parenchyma.

Conclusion: These findings suggest that the B cells interact with the T cells and are involved in antibody secretion by the plasma cells.

Anti-NMDA receptor (NMDAR) encephalitis is thought to be antibody-mediated [1,2]. Brain biopsy or autopsy has shown mild perivascular lymphocytic cuffing and microglial activation [1–4]. Recently, Tüzün *et al*. [5] reported the first immunopathological analysis of the inflammatory reaction in two autopsy cases and found immunoglobulin deposits in the brain. We here describe the histopathology of a brain biopsy indicating that B cells participate in the inflammatory reaction.

Case history

An 18-year-old woman was referred in mid-November 2007 for mood changes, irritability, impaired attention, weight loss and left facial jerks corresponding to epileptic discharges on the electroencephalogram. There was no preceding flu-like illness. The general and neurological examinations were normal. The brain MRI showed small foci of T2 hyperintensities in the right frontal lobe white matter with gadolinium enhancement on T1-weighted sequences. The cerebrospinal fluid (CSF) contained 21 lymphocytes/mm^3 and 0.50 g/l of protein, with 12% oligoclonal IgGs. The neurological status deteriorated, with intractable alternating right and left facial seizures and loss of consciousness, and the patient was transferred to an intensive care unit. With intravenous anti-epileptic drugs, the seizures disappeared. However, the patient was unresponsive to stimulation and required a feeding tube and transient respiratory assistance because of hypoventilation. Repetitive movements of the hands and feet and brisk ocular jerks were present. There was no dysautonomia. As primary angiitis of the central nervous system was discussed, a right frontal lobe biopsy was performed on December 26. Screening for anti-Hu, CV2/CRMP5, Ma2, Yo, Ri, amphiphysin, Tr, lupus, SSA and SSB antibodies was negative. The patient received 5 g of intravenous methylprednisolone, resulting in a dramatic improvement, and she was discharged one week later, but relapsed after one month. As she showed mild improvement after a second bolus of steroids, five monthly pulses of cyclophosphamide were administered, and one year later, she had completely recovered. Two pelvic CT scans in 2008 and 2009 failed to find ovarian teratoma.

Detection of anti-NMDAR antibodies

Antibodies were assessed as described previously [1,2]. Briefly, HEK 293 cells were co-transfected with plasmids coding for NR1 and NR2B fused to GFP, forming NR1–NR2B heteromers of the NMDAR. After 24 h, the cells were fixed briefly in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and incubated

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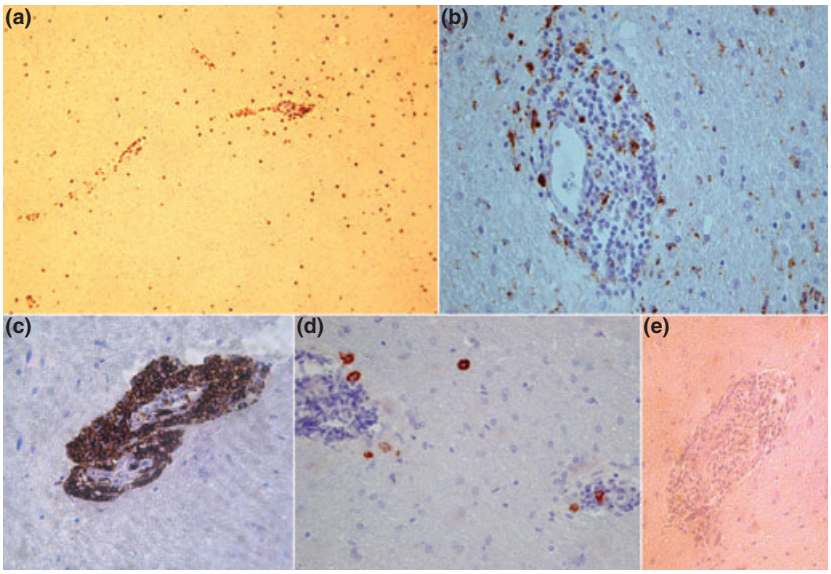

Figure 1 Immunohistochemical study using anti-CD3 (a), CD68 (b), CD20 (c), CD138 (d) and CD1a (e) antibodies. Immunoperoxidase staining. CD3-positive T cells and CD68-positive macrophages are scattered around small vessels and in the parenchyma, whilst there is prominent perivascular cuffing of CD20-positive B cells. Some CD138-positive plasma cells are present. Major histocompatibility complex II–associated CD1a protein is not expressed. All sections were counterstained with haematoxylin. Magnification ×10 (a), ×20 (c and e) and ×40 (b and d).

with the patient's CSF and then with Alexa 488 goat anti-human IgG fluorescent conjugate (Invitrogen, Cergy-Pontoise, France). The patient's CSF was also tested by immunohistochemistry on non-fixed cryocut sections of rat hippocampus [6]. Two CSF samples taken in January 2008, just after the first steroid infusion, and in March 2008, on cyclophosphamide, were tested in September 2009. The first sample was positive at a 1:10 dilution both on the neuropile of the rat hippocampus and on the transfected HEK293 cells. No antibodies were detected in March 2008.

Pathological study

A surgical brain biopsy was performed on the right frontal lobe, and the tissues were fixed in 10% formalin and stained with HE, Bodian and Luxol fast blue. A PCR test was negative for herpes simplex, herpes zoster, HHV6, cytomegalovirus and measles virus. Neuron changes, neuronophagy, axon loss, demyelination and gliosis were absent. Observed changes consisted of marked mononuclear cell accumulation in the arachnoid and around small vessels in both the white and grey matter. In an immunohistochemical study performed using antibodies against CD3, CD68, CD20, CD138, CD1a, glial fibrillary acid protein and Ki67, a few CD3-positive T lymphocytes and CD68-positive macrophages were found scattered throughout the white and grey matter and in the perivascular spaces, whilst the perivascular cuffs were mainly composed of CD20-positive B lymphocytes. In some perivascular spaces, these infiltrates were particularly abundant and only a few B cells were detected in the adjacent parenchyma. CD138-positive plasma cells were comparatively few and were scattered throughout the perivascular space and adjoining parenchyma. CD1a was not expressed, and the Ki67 index indicated that fewer than 5% of the mononuclear cells were dividing (Fig. 1).

Discussion

In this patient, the age, gender, clinical presentation, inflammatory CSF results, MRI changes and response to immunomodulatory treatments were consistent with the now well-established pattern of anti-NMDAR encephalitis [1,7]. Teratoma, which is found in 61% of women with this disorder [1], was not detected, but this does not exclude a microscopic lesion. Retrospective screening for anti-NMDAR antibodies was performed on a CSF sample taken a few days after a massive dose of steroids, which may have contributed to their low level at that time [8]; unfortunately, no serum sample was available.

The brain inflammatory reaction of anti-NMDAR encephalitis has been studied immunopathologically in only two autopsy cases [2,5]. The most prominent features were gliosis, microglial cell proliferation and IgG deposits, whilst mononuclear cells were scarce, consisting of a few perivascular B lymphocytes and plasma cells. In our patient, microglial cells and T cells infiltrated the parenchyma and were scattered around vessels, indicating an encephalitic process. However, compared with previously reported cases, the inflammatory reaction was more intense and B-cell accumulation in the perivascular space was a striking feature. Similar findings have been

reported in cases with anti-VGKC antibodies [9] or classical onconeural antibodies [10]. Anti-NMDAR encephalitis is suspected to be antibody-mediated. Consistent with this hypothesis, Tüzün *et al*. [5] found perivascular plasma cells and immunoglobulin deposits in the brain. In our case, plasma cells were present, but in low numbers. The significance of the B-cell accumulation in the Virchow-Robin spaces, seen in our study, remains unknown. B cells play different role in the pathogenesis of autoimmune neurological disorders, not only as precursors of antibody-producing cells but also as regulators of T-cell activation and the formation of ectopic germinal centres in the intermeningeal spaces [11]. Another distinctive feature of our case was the paucity of gliosis and microglial cell proliferation compared with previously reported cases [1,2,5]. All these findings may be explained by sampling, because the frontal lobe biopsy may not be representative of events elsewhere, which may also be transient. Another factor is that the biopsy was performed before immunosuppressive treatment, which was not the case in the autopsied patients, a fact that may have modified the pattern of the inflammatory reaction.

Taken together, these data suggest that perivascular inflammatory B-cell accumulation may occur in patients with anti-NMDAR encephalitis as part of the immune reaction and may play an active role in brain T-cell infiltration, antibody secretion by plasma cells and microglial and astro-glial proliferation.

Ethics committee

For a single case report using data obtained during the regular management of his/her disease and care without specific investigations performed for a research purpose, the approval of our Hospital Ethics Committee is not requested, but could be obtained if specifically requested.

References

1. Dalmau J, Gleichman AJ, Hughes EG, *et al*. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 2008; **7:** 1091–1098.
2. Dalmau J, Tuzun E, Wu HY, *et al*. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* 2007; **61:** 25–36.
3. Stein-Wexler R, Wootton-Gorges SL, Greco CM, Brunberg JA. Paraneoplastic limbic encephalitis in a teenage girl with an immature ovarian teratoma. *Pediatr Radiol* 2005; **35:** 694–697.
4. Sansing LH, Tuzun E, Ko MW, Baccon J, Lynch DR, Dalmau J. A patient with encephalitis associated with NMDA receptor antibodies. *Nat Clin Pract Neurol* 2007; **3:** 291–296.
5. Tuzun E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J. Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathol* 2009; **118:** 737–743.
6. Ances BM, Vitaliani R, Taylor RA, *et al*. Treatment-responsive limbic encephalitis identified by neuropil antibodies: MRI and PET correlates. *Brain* 2005; **128:** 1764–1777.
7. Iizuka T, Sakai F, Ide T, *et al*. Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. *Neurology* 2008; **70:** 504–511.
8. Seki M, Suzuki S, Iizuka T, *et al*. Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. *J Neurol Neurosurg Psychiatry* 2008; **79:** 324–326.
9. Khan NL, Jeffree MA, Good C, Macleod W, Al-Sarraj S. Histopathology of VGKC antibody-associated limbic encephalitis. *Neurology* 2009; **72:** 1703–1705.
10. Vigliani MC, Novero D, Cerrato P, *et al*. Double step paraneoplastic brainstem encephalitis: a clinicopathological study. *J Neurol Neurosurg Psychiatry* 2009; **80:** 693–695.
11. Dalakas MC. Invited article: inhibition of B cell functions: implications for neurology. *Neurology* 2008; **70:** 2252–2260.