Cerebrospinal fluid CD19+ B-cell expansion in *N*-methyl-D-aspartate receptor encephalitis

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ABBREVIATIONS

CSF	Cerebrospinal fluid
NMDAR	*N*-methyl-D-aspartate receptor

There is increasing interest in the role of autoantibodies in acquired autoimmune central nervous system disorders. *N*-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune encephalitis defined by the presence of autoantibodies that bind to the NMDAR. Although there is evidence of NMDAR antibody pathogenicity, it is unclear which treatment results in the best outcome. We measured the proportion of B-cells in the cerebrospinal fluid of two children with NMDAR encephalitis (a 6-year-old male and a 4-year-old female), one in the acute phase and one in the relapsing phase. The proportion of CD19+ B-cells in both children was greater than 10%, significantly higher than seen in non-inflammatory neurological disorders (<1%). This finding supports the use of drugs, such as rituximab, that deplete B-cells in severe or refractory cases of NMDAR encephalitis, and lends further support to the humoral autoimmune hypothesis in NMDAR encephalitis.

There is increasing interest in the role of central nervous system autoimmunity mediated by B-cells and autoantibodies. Antibodies that bind to neuronal receptors and synaptic proteins are increasingly important biomarkers and mediators of acquired neurological and psychiatric disease.[1,2]

N-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune encephalitis that affects children and young adults, and is defined by serum and cerebrospinal fluid (CSF) autoantibodies against the NR1 subunit of the NMDAR. There is already clear evidence that NMDAR antibodies have a pathogenic role and down-regulate the receptor from the neuronal cell surface.[1,3] Some pathological studies have shown that the parenchymal inflammatory infiltrate in perivascular regions has an excess of plasmablasts and CD138 plasma cells,[4] although a separate report has shown a low density of inflammatory cells and very limited neuropathology.[5]

CSF immunophenotyping has demonstrated B-cell expansion in opsoclonus–myoclonus and neuromyelitis optica, and has been used to support the humoral autoimmune hypothesis, and the use of drugs that deplete B-cell populations such as rituximab.[6,7]

We present our findings of CSF B-cell expansion in a child with acute NMDAR encephalitis and in another child with relapsing NMDAR encephalitis. Both children had substantial expansion of B-cells in the CSF, a finding that supports the use of drugs such as rituximab that target CD20+ B-cells.

CASE REPORTS

Method

Lymphocyte immunophenotyping was performed using a previously described protocol.[6] CSF samples (range 10–500μL with a red blood cell count <100 red blood cells per mm^3) were collected and immediately placed on ice, and then centrifuged at 600*g* for 7 minutes at 4°C. It was usually possible to perform CSF immunophenotyping with less than 1mL of CSF. Non-specific Fc receptor binding was blocked with 0.2mg/mL normal mouse immunoglobulin G (Invitrogen, Carlsbad, CA, USA) for 15 minutes at room temperature. Directly labelled anti-CD3 (clone UCHT1; BD Biosciences, Sparks, MD USA), anti-CD19 (clone SJ25-C1; Invitrogen), anti-CD4 (clone RPA-T4; BD Biosciences), anti-CD8 (clone SK1; BD Biosciences), anti-CD27 (clone L128; BD Biosciences), anti-CD14 (clone TUK4; Invitrogen), anti-CD138 (clone MI15; BD Biosciences), and anti-CD38 (clone HIT2; eBioscience, San Diego, CA, USA) were added to the cells without washing the mouse immunoglobulin G. The samples were incubated for 15 minutes at room temperature in the dark. Cells were washed once with 1mL of ice-cold phosphate-buffered saline supplemented with 2% fetal bovine serum (Invitrogen) and centrifuged at room temperature for 30 seconds at 5000*g* in a table-top microcentrifuge. The supernatant was carefully removed by pipetting. Cells were then resuspended in 100μL of phosphate-buffered saline and fetal bovine saline and 1% paraformaldehyde. All cells in the sample were then acquired on a BD LSRII instrument (BD Biosciences), and data were analysed with FlowJo software (TreeStar, Ashland, OR, USA).

Using this approach, Pranzatelli et al.[8] found that children with non-inflammatory neurological disorders such as myoclonus, ataxia, intracranial hypertension, chronic daily headache, and developmental delay all had a low proportion of

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$CD19^+$ B-cells in CSF (<1%). Similarly, we have also shown that in children (mean age 7y; range 2–15y, presented in detail in a previous report)[6] with non-inflammatory neurological disorders such as hydrocephalus, Tourette syndrome, epilepsy, and dystonic cerebral palsy the proportion of $CD19^+$ B-cells in CSF is low (<1%).[6] The protocol for this report was approved by the Children's Hospital at Westmead Human Ethics Committee, and written consent was acquired from the patients' parents for CSF immunophenotyping and for reporting the cases.

Case 1

A previously well 6-year-old Vietnamese male presented with a 1-week history of altered behaviour, sleep disturbance, and involuntary limb movements. The abnormal behaviour included episodic agitation, repetitive questioning, and inappropriate behaviour. He experienced focal seizures with dyscognitive features confirmed using video telemetry. Initial clinical examination revealed an agitated state plus intermittent dystonia and chorea of the limbs. CSF analysis revealed three polymorphs, six mononuclear cells, three red blood cells with normal protein, negative oligoclonal bands, and marginally elevated neopterin of 32nmol/L (normal <28nmol/L).[9] Examination of acute CSF on admission and before therapy revealed $CD19^+$ B-cell expansion of 10.2% (normal <1%; Fig. 1a and b). CSF $CD4^+$ and $CD8^+$ percentages were not different from those previously recorded in children with other neurological disorders (62% $CD4^+$ and 27.4% $CD8^+$ vs 64.16% $CD4^+$ [SD 5.5%] and 25.2% $CD8^+$ [SD 6.6%];[6] Fig. 1a and b). Concomitant blood $CD19^+$ B-cell percentages were normal (25%; normal 10–31%). Electroencephalography on admission revealed diffuse slowing during wakefulness with left hemispheric epileptiform discharges and brain magnetic resonance imaging (MRI) was normal. The patient was commenced on cefotaxime, acyclovir, and phenytoin. Both serum and CSF were positive for NMDAR antibodies (national referral laboratory), and the patient was commenced on intravenous methylprednisolone (30mg/kg/d) for 3 days along with intravenous immunoglobulin (2g/kg) over 2 days. The dominant clinical features during admission were episodic confusion and agitation along with insomnia and daytime somnolence. The limb dystonia and chorea improved within 10 days of commencing therapy. Two months after the development of illness the patient has made a complete physical recovery and his abilities are back to baseline level; however, he remains emotionally labile and inattentive. He is on a weaning dose of oral prednisolone (initially 2mg/kg/d, weaning over 3mo) and antiepileptic medication.

Case 2

A previously well 4-year-old white Australian female presented in 2007 with a subacute onset of emotional lability, focal seizures, altered speech, and chorea. Her CSF showed no pleocytosis, but intrathecal oligoclonal bands and elevated neopterin of 115nmol/L (normal <28nmol/L), although MRI was normal. An autoimmune encephalitis was suspected and she responded to intravenous methylprednisolone 30mg/kg/day for 3 days and intravenous immunoglobulin 2g/kg given over 2 days. The patient made a good recovery, and a retrospective diagnosis of NMDAR encephalitis was made using saved acute serum in 2009 (Oxford, UK).[10] The patient had a relapse with recurrence of hemidystonia and speech disturbance in 2010, which again responded to steroids and immunoglobulin. A further relapse a year later was characterized by word-finding difficulties, emotional lability, and focal seizures confirmed using video telemetry. Sequential pelvic ultrasound examinations and MRI of the pelvis have been normal, as has MRI of the brain. During the latest relapse the CSF showed four monocytes and intrathecal oligoclonal bands, but normal neopterin. CSF and serum NMDAR antibodies during the relapse were positive (in-house assay and national referral laboratory). Fresh CSF was used for CSF immunophenotyping. The patient had significant B-cell expansion with 10.7% $CD19^+$ B-cells (controls <1%; Fig. 1a and b). There were not enough cells to count the number of plasma cells. The proportions of $CD4^+$ and $CD8^+$ in CSF were not different from those in comparison children without neurological disorders (61.2% $CD4^+$ and 30% $CD8^+$; Fig. 1a and b). Concomitant blood $CD19^+$ B-cell percentages were normal (30%; normal 10–31%). The patient again responded to intravenous methylprednisolone 30mg/kg/day for 3 days and oral prednisolone (2mg/kg/d, weaning over 3mo), and has been immune suppressed with mycophenolate mofetil (750mg twice a day). She has been in remission for 9 months, with a return to baseline ability levels.

What this paper adds

- We provide evidence of B-cell expansion in the cerebrospinal fluid of patients with NMDAR encephalitis.
- This finding supports a possible therapeutic role of rituximab.

DISCUSSION

This is the first examination of lymphocyte immunophenotyping in the CSF of patients with NMDAR encephalitis. In both patients there was very significant B-cell expansion, similar to or higher than that described in cases of opsoclonus–myoclonus syndrome, a putative autoimmune disorder that can be treated with rituximab.[7] This finding supports the role of CSF humoral autoimmunity in both the acute and relapsing course of NMDAR encephalitis. Given the fact that many patients with NMDAR encephalitis exhibit intrathecal synthesis of NMDAR antibody, B-cell expansion and differentiation would be anticipated and expected.[1] CSF B-cell expansion has been used to support the use of rituximab. However, it should be noted that rituximab depletes $CD20^+$ B-cells, but not $CD138^+$ antibody-producing plasma cells (which are CD20 negative). Indeed, the mechanism of action of rituximab is likely to be complex, as has recently been shown in multiple sclerosis.[11] Demonstrating CSF B-cell depletion after treatment of NMDAR encephalitis with rituximab would strengthen the importance of our finding.

The best second-line therapy for severe, refractory, or relapsing NMDAR encephalitis is unclear, although mycophenolate mofetil, rituximab, and cyclophosphamide have all

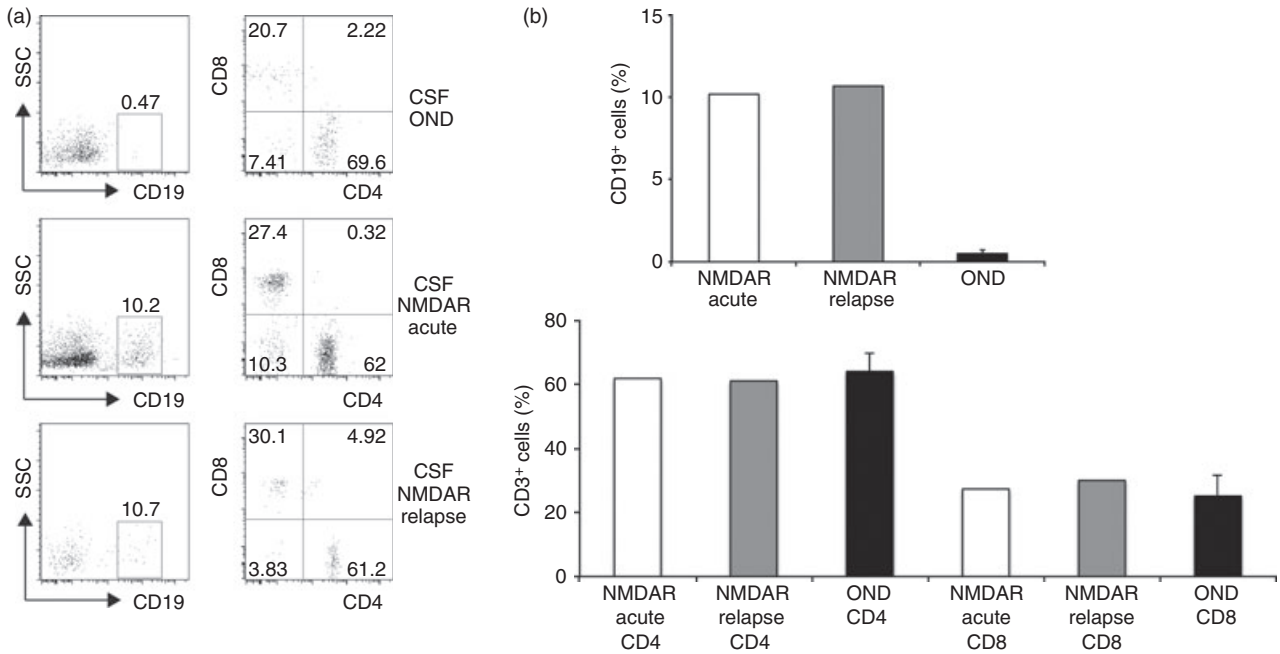

Figure 1: B- and T-cell immunophenotyping of cerebrospinal fluid (CSF) from children with acute and relapsing *N*-methyl-D-aspartate receptor (NMDAR) encephalitis and other neurological disorders (ONDs; $n = 5$). (a) Flow cytometry dot plots of $CD19^+$ B-cells (left) and $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T-cells (right) in CSF from case 1 (acute NMDAR encephalitis) and case 2 (relapsing NMDAR encephalitis). B-cell percentages were increased in both acute and relapse NMDAR encephalitis, whereas $CD4^+$ and $CD8^+$ T-cell percentages were similar in all patients. (b) Analysis of the proportion of B-cells ($CD19^+$, top bar graph) and T-cells ($CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$, bottom bar graph) in the CSF of case 1 (acute NMDAR) and case 2 (NMDAR relapse) and in five individuals with OND. SSC, side scatter.

been used with success. We have elected to treat the child with a relapsing course with a steroid-sparing immune suppressant (mycophenolate mofetil) in the first instance, and we will use rituximab if this is ineffective. Further study of lymphocyte characteristics in the CSF such as clonal expansion may be informative, as has been shown in neuromyelitis optica.[12]

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