RESEARCH ARTICLE

N-Methyl-D-Aspartate Receptor Antibodies in Post–Herpes Simplex Virus Encephalitis Neurological Relapse

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ABSTRACT: Herpes simplex virus encephalitis (HSVE) is a devastating condition that relapses, often with a chorea in children, despite adequate antiviral treatment. At relapse, evidence of viral replication is frequently absent, suggesting that the relapse may be immune-mediated. Seven children who had a neurological relapse following their initial encephalitis, identified from 20 cases of pediatric HSVE, were studied. Serum and/or cerebrospinal fluid (CSF) were tested for N-methyl-D-aspartate receptor (NMDAR) and other antibodies previously reported in central nervous system autoimmunity. Five of the 7 relapsing children had choreoathetosis; 2 of these were NMDAR antibody–positive, 2 were negative (1 with HSV-positive CSF), and 1 was not available for testing. An additional patient, who relapsed with cognitive regression but with no movement disorder, was also NMDAR antibody–positive. In 2 of the NMDAR antibody–positive patients who were treated at relapse and in 1 who was treated following only after 10 years of having a relapsing encephalopathy, a beneficial response was observed. Neurological relapses after HSVE may frequently be immune-mediated, particularly in children with chorea. NMDAR antibodies are common, and immunotherapy may be beneficial. © 2013 Movement Disorder Society

Key Words: herpes simplex virus; encephalitis; N-methyl-D-aspartate (NMDA) receptor; choreoathetosis; movement disorder; relapsing

Herpes simplex virus encephalitis (HSVE) is associated with significant morbidity and mortality.1 Optimal therapy of HSVE relies upon good central nervous system (CNS) delivery of acyclovir and adequate duration of treatment, with current recommendations being 14 to 21 days of intravenous (IV) acyclovir at 1500 mg/m²/d.2

Additional Supporting Information may be found in the online version of this article.

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Despite this, patients with HSVE can relapse with a second phase of encephalopathy with fever, seizures, and often with a florid choreoathetoid movement disorder. At relapse, patients are often assumed to have an acute infective relapse prompting longer treatment courses of antiviral agents, although evidence of viral reactivation has been found in less than 25% of adults and children (Supplemental Table 1), suggesting that the relapses could be an immune-mediated mechanism, as proposed. There are now a number of distinct neurological syndromes in which brain-directed antibodies have been identified, with a significant number of conditions featuring movement disorders. In particular, the movement disorder seen in N-methyl-D-aspartate receptor (NMDAR) antibody encephalitis, a well-characterized encephalopathy syndrome with prominent psychiatric symptoms, seizures, movement disorders, and autonomic instability, has phenotypic similarities to the choreoathetosis in relapsing HSVE. NMDAR antibodies have been detected in a proportion of adult patients with acute HSVE, and in 20 Spanish children with anti-NMDAR encephalitis, had presented as choreoathetosis post-HSVE. These and other evidence of viral infections coincident with NMDAR antibodies in adults and children, raise the possibility that induction of NMDAR antibodies during the viral infection may be responsible for a second phase of immune-mediated encephalopathy.

We reviewed reports of relapses in children and adults (Supplemental Table 1), and studied 7 children who relapsed with a further encephalopathic episode following HSVE. We identified NMDAR antibodies in half of the 6 available samples from these patients.

Patients and Methods

Seven children (6 months to 15 years old) who had relapsed following their initial presentation were retrospectively identified out of the 20 cases of HSVE studied at the Evelina Children’s Hospital (ECH) (London, UK; n = 9) and Bicêtre Hospital (BH) (Paris, France; n = 11), between 2000-2012 and 2006-2012, respectively. Clinical information and neuroimaging were reviewed (Y.H., A.S., M.L., and K.D.).

The 5 available sera and 1 cerebrospinal fluid (CSF) were tested for immunoglobulin G (IgG), IgA, IgM, and IgE antibodies to the NMDAR using a cell-based assay, with subtype-specific goat anti-human secondary antibodies (Alexafluor 568 goat anti-human IgG [Molecular Probes, Eugene, OR, USA]; or goat anti-human IgA, IgM, or IgE [Vector, Burlingame, CA, USA]; 1:750), and a third layer of fluorescein-conjugated rabbit anti-goat IgG (Molecular Probes, Eugene, OR, USA; 1:750) for IgA, IgM, and IgE visualization. Positive samples were tested at serial dilutions until endpoints were reached. Antibody binding to primary cultures of mouse hippocampal neurons (sera 1:200) and rat brain sections (sera 1:200, CSF 1:10) were used to support serum antibody positivity, particularly in the absence of CSF availability. All assays were assessed by 2 independent observers (Y.H. and P.P.).

Results

The clinical and immunological features of the 7 patients with a second encephalopathic episode following HSVE are summarized in Table 1. The children initially presented with clinical, laboratory, and radiological findings consistent with HSVE and had made a significant clinical improvement with IV acyclovir at 30 mg/kg (500 mg/m²) per dose every 8 hours for 21 days. Three cases (ECH cases 2, 3, and 4) were then given long-term valacyclovir. At relapse (16-36 days after initial presentation), all patients were encephalopathic, with additional choreoathetoid movement disorder in 5 (71%), seizure disorder in 4 (57%), and cognitive regression in 2 (29%). Viral reactivation was identified in only 1 patient (14%). For the remaining 6 patients with no evidence of viral reactivation, a range of infective and alternative inflammatory causes were sought but not identified at the time.

Overall, serum IgG NMDAR antibodies, but not the IgA, IgM, or IgE subtype, were detected in both of the 2 patient samples tested at relapse (BH case 1 and BH case 2), and in 1 (of 3) patients who was tested 10 years from her initial relapse during the course of her relapsing disease (ECH case 3); endpoint titers were 1:540 or greater (Table 1). One additional CSF (ECH case 2) was negative after relapse and no samples were available from BH case 3. All 3 patients with positive NMDAR antibodies were acutely or subacutely symptomatic at the time of sampling, in contrast to 1 of 3 who were antibody-negative. The 3 NMDAR antibody-positive sera showed the typical neuropil NMDAR antibody staining of the hippocampus on rat brain tissue sections (see Fig. 1G, H for BH case 2) and sera from 2 patients (BH case 2; Fig. 1I and ECH case 3) bound to the surface of live hippocampal neurons; insufficient serum was available for testing from BH case 1. When sera and/or CSF were sufficient, further testing of 5 patients (no samples for ECH case 2 and BH case 3) did not reveal antibodies to voltage gated potassium channel (VGKC)-complex (including the associated proteins leucine-rich glioma-inactivated 1, LGI1; and contactin associated protein 2, CASPR2), glycine or dopamine 1 and 2 receptors (D1R and D2R).

Only children presenting to BH were treated empirically with immunotherapy at relapse, reflecting institutional differences in management. In all 3 patients with NMDAR antibodies, a convincing beneficial response was observed in BH cases 1 and 2, and a probable early response following delayed treatment 12 years later in ECH case 3. Here we describe BH case 2, ECH case 3, and BH case 1. The NMDAR
**TABLE 1.** Children with relapsing neurological syndromes after HSV encephalitis

<table>
<thead>
<tr>
<th>Case (year)</th>
<th>Age/sex</th>
<th>HSV PCR at onset</th>
<th>NMDAR Ab at onset</th>
<th>Relapse after presentation</th>
<th>Neurological syndrome at relapse</th>
<th>HSV PCR at relapse</th>
<th>NMDAR Ab at relapse</th>
<th>Current NMDAR Ab; time since relapse</th>
<th>Immunoresponse; response</th>
<th>Current neurological condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECH case 1</td>
<td>7 months/M</td>
<td>+</td>
<td>N/A</td>
<td>Day 27</td>
<td>Encephalopathy; seizures</td>
<td>−</td>
<td>N/A</td>
<td>Negative serum (CBA, HN, IHC); 11 years</td>
<td>Not treated</td>
<td>Residual motor and cognitive deficit</td>
</tr>
<tr>
<td>ECH case 2</td>
<td>20 months/F</td>
<td>+</td>
<td>N/A</td>
<td>Day 35</td>
<td>Encephalopathy; movement disorder (OFD, C, A)</td>
<td>−</td>
<td>N/A</td>
<td>Negative CSF (CBA, IHC); 10 years</td>
<td>Not treated</td>
<td>Residual motor and cognitive deficit</td>
</tr>
<tr>
<td>ECH case 3</td>
<td>10 months/F</td>
<td>+</td>
<td>N/A</td>
<td>Day 40³</td>
<td>Encephalopathy; movement disorder (OFD, C, A), cognitive regression</td>
<td>−</td>
<td>N/A</td>
<td>Positive serum (CBA 1:1620, HN, IHC); MG: improvement in cognition, speech, and seizure control</td>
<td>Residual motor and cognitive deficit³</td>
<td></td>
</tr>
<tr>
<td>ECH case 4</td>
<td>6 months/M</td>
<td>+</td>
<td>N/A</td>
<td>Day 18³</td>
<td>Encephalopathy; movement disorder (OFD, C), seizures</td>
<td>+</td>
<td>N/A</td>
<td>Negative serum (CBA, HN, IHC); 10 years</td>
<td>Not treated</td>
<td>Residual motor deficit</td>
</tr>
<tr>
<td>BH case 1</td>
<td>15 years/F</td>
<td>+</td>
<td>N/A</td>
<td>Day 36</td>
<td>Encephalopathy, cognitive regression</td>
<td>−</td>
<td>Positive serum (CBA 1:540, IHC)²</td>
<td>N/A</td>
<td>MMP cyclosporine: improvement with resolution of encephalopathy</td>
<td>Residual cognitive deficit</td>
</tr>
<tr>
<td>BH case 2</td>
<td>3 years/F</td>
<td>+</td>
<td>Negative serum (CBA, HN, IHC)</td>
<td>Day 31</td>
<td>Encephalopathy; movement disorder (OFD, C, A, S), seizures</td>
<td>−</td>
<td>Positive serum (CBA 1:1620, HN, IHC); Positive serum (Euroimun 1:10000)</td>
<td>N/A</td>
<td>MMP, MMF, rituximab: resolution of encephalopathy and movement disorder</td>
<td>Residual cognitive deficit</td>
</tr>
<tr>
<td>BH case 3</td>
<td>9 months/M</td>
<td>+</td>
<td>N/A</td>
<td>Day 36</td>
<td>Encephalopathy; movement disorder (C, A), seizures</td>
<td>−</td>
<td>N/A</td>
<td>N/A</td>
<td>MMP, PLEX, MMF: full recovery</td>
<td>Age-appropriate development</td>
</tr>
</tbody>
</table>

*Patients treated with intravenous acyclovir regime at both institutions; 30 mg/kg (500 mg/m²)/dose every 8 hours for 21 days.

*Patients relapsed while on antiviral therapy.

*Patient continues to have intermittent episodes of cognitive regression unrelated to seizure disorder.

*Patient serum taken 12 months after relapse while on immunotherapy.

A 3-year-old girl presented acutely with fever, vomiting, headache, and progressive encephalopathy evolving over 2 days. CSF revealed pleocytosis and raised protein, and IV acyclovir was commenced. Her neuroimaging was suggestive of HSVE (Fig. 1A-C). Subsequently, a positive HSV polymerase chain reaction (PCR) was confirmed in CSF. She improved clinically on completion of 21 days of IV acyclovir therapy. Ten days later, she became encephalopathic again with behavioral change and choreoathetoid movements. She additionally had facial dyskinesia and numerous nonpurposeful movements. No HSV reactivation in CSF was detected. Her electroencephalogram demonstrated diffuse generalized slow activity. Repeat neuroimaging showed evolution of her frontotemporal lesions with necrosis and volume loss in the right temporal lobe (Fig. 1D-F). Acyclovir was restarted with the addition of high-dose steroids. Following 15 days of treatment she went on to receive plasmapheresis but this was stopped after 3 cycles due to nontolerance. Persistence of the clinical signs and the resemblance of the movements to patients with anti-NMDAR encephalitis prompted antibody testing that was positive in serum (1:1620), whereas the initial serum sample at the onset of her HSVE was negative for NMDAR antibodies. Oral treatment with mycophenolate mofetil (MMF) was commenced 5 weeks into her relapse when she had remained bedridden with chorea. At day 15 of MMF treatment, an improvement in the encephalopathy was seen; at day 21 the movement disorder started to improve and she was able to walk again; and by 3 months her speech had returned. At 1-year follow-up, she was able to attend school but her behavior remained challenging with hyperactivity and disinhibition. Two years after her relapse she is clinically stable, but NMDAR antibodies remain high in her serum (1:1000 Euroimun tested locally), and in view of ongoing behavioral problems, rituximab has been added.

**ECH Case 3**

A 10-month-old girl presented with prolonged seizures and progressive encephalopathy. Her electroencephalogram (EEG) and neuroimaging (CT) revealed temporal lobe pathology (Fig. 2A, B), prompting early empirical treatment of HSVE. Magnetic resonance imaging (MRI) revealed extensive area of left temporal, parietal, and occipital cortical destruction (Fig. 2C, D). The clinical and radiological diagnosis was...
confirmed with a positive HSV PCR detected in CSF. The patient made an uncomplicated neurological recovery and was discharged on long-term valacyclovir, a treatment strategy used at this institution for all young patients with focal destructive HSV. Five days later (day 40 from first presentation), she presented again with a florid dyskinetic movement disorder associated with progressive loss of skills. She had prominent orofacial (and lingual) dyskinesia, widespread chorea with distal athetosis, and asymmetric ballistic movements which were more prominent on the left, lateralizing to her less-affected hemisphere as seen on neuroimaging. She was managed with a second course of intravenous acyclovir. In the subsequent 10 years, after an initial apparent good recovery that included independent ambulation and hand function in the second and third years of life, she suffered recurrent episodes (on average twice a year lasting for a few weeks) of cognitive and behavioral decline with worsening seizures, speech regression, and food refusal, assumed to be sequelae of her initial HSVE. A recent reevaluation of the clinical history and video footage prompted further investigations to explore the possibility of a fluctuating immune encephalopathy, which identified a strong NMDAR antibody positivity detailed in Table 1. CSF analysis also revealed intrathecal synthesis of oligoclonal bands. A temporal correlation between her clinical picture and antibody positivity was observed with titers of 1:1620 when symptomatic and reducing to 1:540 in between...

FIG. 1. Neuroimaging of BH case 2 (A–F). Axial FLAIR images showing an area of parenchymal hyperintensity involving the right temporal lobe (dashed arrow, A) extending into the adjacent frontoparietal regions (dashed arrow, B) and insula (dashed arrow, C), with some early involvement in the contralateral temporal lobe. Repeat MRI on day 30 demonstrates progression of hyperintensity to involve larger regions of the left and right temporal lobes (white solid arrows, D), frontoparietal white matter (white arrows, E), and insula (white arrows, F) with ex vacuo enlargement of the left lateral ventricle and less marked encephalomalacia of the mesial temporal lobe (D, E). This characteristic limbic involvement with apparently unilateral onset and subsequent less marked contralateral limbic involvement (“sequential bilaterality”) is consistent with HSVE. Detection and characterization of NMDAR antibodies in BH case 2 (G–I). Hippocampal “neuropil” binding pattern on sagittal rat brain sections (G, ×10) and the dentate gyrus shown in higher magnification (H, ×20). IgG NMDAR-antibody-positive serum (green) showing surface binding to two superimposed neurons and their processes in primary cultures of live hippocampal neurons identified by the neuronal marker microtubule–associated protein 2 (MAP2, in red) (I, ×100). Cell-based assay for NR1/NR2B (NMDAR) antibodies using transfected human embryonic kidney (HEK293) cells shows surface binding (red) (J, ×40).
episodes of encephalopathy. In view of the antibody positivity, she was recently started on treatment with 4 weekly intravenous immunoglobulins (IVIG). Clinical improvement in cognition, speech, and seizure control was observed by both parents and school after the first course and is sustained 3 months later.

**BH Case 1**

A 15-year-old girl presented with headaches, fever, behavioral change, and alteration of consciousness. Cranial MRI revealed cortical and subcortical T2 signal changes in the left temporal lobe and bilateral frontal lobes with additional changes in the right hippocampus and insula (Fig. 3A, B). CSF analysis demonstrated pleocytosis (white cell count 920) and IV acyclovir was commenced. HSV infection was confirmed by positive PCR in the CSF. She was discharged home with near complete recovery. At day 60 (21 days after treatment was stopped), she re-presented with severe headaches, concentration difficulties, sleepiness, and weight loss, and on clinical assessment was found to be disinhibited with signs of meningism. MRI revealed extensive white matter lesions involving the frontal, temporal, and parietal lobes bilaterally (Fig. 3C, D). CSF showed 3 white cells with elevated protein of 1.38 g/L and negative HSV PCR. Her clinical symptoms continued to deteriorate, with progression of neuroimaging (Fig. 3E, F) and she developed raised intracranial hypertension. High doses of methylprednisolone followed by cyclosporine was given at day 65 (cyclosporine continued for 1 year) and a significant clinical improvement was noticed, with resolution of the encephalopathy 3 weeks following initiation of treatment and a return to school after 2 months. By 3 years, she had made a good recovery, but her behavior remains challenging requiring psychiatric input. Long-term follow-up imaging demonstrates resolution of changes with volume loss (Fig. 3G, H). A sample taken while she was on immunotherapy was retrospectively tested revealing anti-NMDAR positivity at titer 1:540.

**Discussion**

Relapses in HSVE were found in 7 of 20 cases of HSVE (35%), somewhat higher than in the reviewed pediatric cases (21%, Fisher’s exact test, $P = 0.09$; Supplemental Table 1), and 5 of these children had a movement disorder, typical of the post-HSVE chorea.⁵ We found NMDAR antibodies in 3 of the 6 children in whom viral reactivation was not identified, and who had relapsed with an encephalopathic syndrome characterized by choreoathetosis, seizures, and/or cognitive regression. Movement disorders are common in pediatric HSVE relapses, and the phenotype of 2 of our NMDAR antibody–positive cases (ECH case 3 and BH case 2) was typical of anti-NMDAR encephalitis.⁸ Although the phenotype in 1 patient at onset of relapse (BH case 1) did not fully resemble that of the anti-NMDAR encephalitis as originally described,⁸ the significant improvement in this patient following immunotherapy suggests that the NMDAR antibodies (positive at 1:540) could have mediated the relapses.

This study was limited by small numbers and its retrospective nature, and consequently sera at various
time points were not available to assess when the induction of antibodies occurred in relation to HSVE, or to the onset of particular encephalopathic features at relapse, and only 1 CSF sample was available for testing (Table 1). In 2 patients who relapsed, 1 with seizures and the other with a movement disorder, NMDAR antibodies were not identified in the sera but this may reflect the late time of sampling, after clinical stabilization, or the lack of availability of CSF for analysis. However, it is possible that another antibody (or immune process) could have mediated their neurological relapse.

NMDAR antibodies of immunoglobulin subtypes IgA, IgG, or IgM were recently detected in the acute samples of 13 of 44 (30%) adult patients with HSVE, but relapses were not reported; indeed, movement disorders, typical of anti-NMDAR encephalitis, were not reported in any of the adult patients reviewed (Supplemental Table 1). IgA NMDAR antibodies have been found in adults with slow cognitive impairment, but although 2 of the patients did relapse with encephalopathy with cognitive regression, we did not find IgA NMDAR antibodies. NMDAR antibodies have also been identified retrospectively in patients with acute encephalitis in whom autoimmunity was not initially considered, and we recently reported HSV PCR positivity in 2 patients with immunotherapy-responsive NMDAR antibody encephalitis who were studied as part of a cohort of 48 children presenting with probable autoimmune encephalitis.

It is becoming clear that the relationship between viral infections and autoimmunity of the central nervous system is complex and needs systematic study in children with the whole spectrum of encephalitis. Inborn errors of innate immunity have been shown to be a susceptibility factor for childhood HSVE, but have not been linked to neurological relapses or to NMDAR antibody encephalitis. Nevertheless, in the 3 children with HSVE relapses who did have NMDAR antibodies, it seems very likely that the viral infection induced NMDAR antibodies, which then led to a second phase of immune-mediated encephalopathy, as recently reported in another child with features typical of anti-NMDAR encephalitis, similar to 2 of our cases. Importantly, in the handful of previous cases of relapsing HSVE, when immunotherapy used was of adequate intensity and duration, a benefit was reported (see also Supplemental Material), as seen in our 3 NMDAR-antibody patients. Although the eventual outcome may be confounded by the neurodevelopmental sequelae of the initial infective encephalitis, rather than that of the relapse, when patients relapse following HSVE in the absence of viral reactivation, an autoimmune (particularly NMDAR antibody-mediated) etiology is an important differential, with investigative and treatment strategies needing to be directed appropriately.

**FIG. 3.** Neuroimaging of BH case 1 (A-H). T2-weighted FLAIR images at presentation (A, B) showing typical HSVE involvement of the limbic lobes with high signal change in the insular cortex bilaterally and asymmetrically (black arrows) and cingulate gyrus (white arrow). Images at the onset of relapse (C, D) show shrinkage of the previously affected limbic cortex but development of new confluent white matter signal abnormality in a similar distribution. Further follow-up scan (E, F) shows progression of the white matter changes that are now associated with cerebral swelling. Long-term follow-up (G, H) demonstrates marked resolution with volume loss and some residual changes.
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