ANTI-*N*-METHYL-D-ASPARTATE RECEPTOR ENCEPHALITIS IN A YOUNG CHILD WITH HISTOLOGICAL EVIDENCE ON BRAIN BIOPSY OF COEXISTENT HERPES SIMPLEX VIRUS TYPE 1 INFECTION

Mark A. Ellul, MRCP(UK), Michael J. Griffiths, MRCPCH,*† Anand Iyer, MD,† Shivaram Avula, FRCR,† Sylviane Defres, MB ChB,* Atik Baborie, FCRPath,‡ Angela Vincent, FRS,§ Natalie G. Martin, MB ChB,¶ Manish Sadarangani, Dphil,¶ Andrew J. Pollard, FRCPCH, PhD,¶ Tom Solomon, FRCP, PhD,*‡ and Rachel Kneen, FRCPCH*†*

Abstract: We report a 3-year-old boy with anti-*N*-methyl-D-aspartate receptor encephalitis with a typical syndrome of movement disorder and encephalopathy and evidence of herpes simplex virus (HSV) type 1 infection on brain biopsy. HSV type 1 infection and anti-*N*-methyl-D-aspartate receptor encephalitis are temporally linked in some cases: this case suggests that prodromal HSV type-1 infection may be clinically subtle and easily missed.

Key Words: encephalitis, encephalopathy, autoantibody, *N*-methyl-D-aspartate receptor, herpes simplex virus

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From the *Institute of Infection and Global Health, University of Liverpool; †Alder Hey Children's NHS Foundation Trust; ‡The Walton Centre NHS Foundation Trust, Liverpool, United Kingdom; §Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital; and ¶Department of Paediatrics, University of Oxford, and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

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Address for correspondence: Rachel Kneen, MRCPCH, Institute of Infection & Global Health, Ronald Ross Building, 8 West Derby Road, Liverpool L69 7BE, United Kingdom. E-mail: rachel.kneen@liverpool.ac.uk.

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Anti-NMDAR encephalitis is a recently described autoimmune disorder of the central nervous system with a recognizable phenotype consisting of cognitive and psychiatric features, movement disorder and progressively deteriorating encephalopathy. It was first described as a paraneoplastic condition in a series of young women with ovarian teratomas, but more recently has been described in children, who are more likely than adults to have had a prodromal illness before presentation and less likely to have an underlying tumor.[1] The outcome is generally good especially if recognized and treated early with immunosuppressant medication.[1] In this report, we describe a child who had anti-NMDAR encephalitis with a classical presentation and a good outcome but also had histological evidence of HSV type 1 infection in neurons.

CASE REPORT

A previously well 3-year-old Caucasian boy presented with a 2 week change in behavior, irritability and altered sleep pattern followed by difficulty swallowing, drooling, encephalopathy and involuntary limb and facial movements. Four weeks earlier, he had a mild encephalopathy, consisting of lethargy and excessive sleepiness, with vomiting lasting 3 days. The parents had sought medical attention, and he was given a course of antibiotics for presumed tonsillitis but had apparently made a full recovery. He had developed a mild fever and coryzal symptoms just before admission. On examination, he was afebrile and encephalopathic with a Glasgow Coma Scale of 9 out of 15 with tachycardia, hyperhidrosis and excessive salivation. He had evidence of right otitis externa but no other signs of infection. He had a florid movement disorder characterized by orofacial dyskinesia and coarse writhing movements of all 4 limbs. He was treated empirically with intravenous aciclovir (total 10 days) and ceftriaxone (total 7 days). His condition deteriorated rapidly over several hours, with periods of agitation alternating with apneic episodes and reduced conscious level with tachycardia and excessive salivation. He was intubated and transferred to a pediatric intensive care unit.

Initial blood workup was normal and blood cultures were negative. Cerebrospinal fluid (CSF) analysis demonstrated 12 white cells/mm^3 (100% lymphocytes), protein 58 mg/dL and glucose 38 mg/dL with CSF/plasma glucose ratio of 0.7. CSF microscopy and culture and polymerase chain reaction (PCR) for HSV types 1 and 2, enterovirus, echovirus and parechovirus were negative. Oligoclonal bands were present in serum and CSF with additional bands in CSF, implying a systemic response with an additional central nervous system-restricted response. Magnetic resonance imaging (MRI) of the brain showed evidence of T2 hyperintensity, diffusely involving the right temporal lobe cortex with right hippocampal volume loss and subtle hyperintensity involving the left amygdala (see Figure A–C, Supplemental Digital Content 1, http://links.lww.com/INF/C340). Electroencephalography demonstrated generalized slowing, most marked over the right cerebral hemisphere, consistent with encephalopathy, without epileptiform features.

A clinical diagnosis of anti-NMDAR encephalitis was made and 500 mg of intravenous methylprednisolone was given once daily on days 5–7 of admission followed by intravenous immunoglobulin (IVIg): total dose 2 g/kg. He was extubated after 5 days, but his conscious level continued to be reduced and the florid movement disorder continued.

Given the unusual MRI findings, which were consistent with HSV type 1 encephalitis, lesional brain and meningeal biopsies were performed on day 10 after admission (see Figure E–I, Supplemental Digital Content 1, http://links.lww.com/INF/C340). These showed a nonspecific active chronic inflammation with occasional polymorphonuclear cells, epithelioid and CD68-positive foamy macrophages, LCA-positive lymphocytes, a few rod and plasma cells and multinucleated giant cells in meninges, white and cortical type grey matter, consistent with meningoencephalitis. The brain parenchyma had a positive PCR for HSV type 1, confirmed on repeat testing, and immunohistochemistry for HSV type 1 showed occasional very faint cytoplasmic staining in neuronal-type cells (see Figure I, Supplemental Digital Figure 1, http://links.lww.com/INF/C340, positive control Figure 1J). Aciclovir was recommenced and given intravenously for a further 21 days. Anti-NMDAR antibodies (IgG), drawn on day 3 of admission, were reported on day 11 of admission to be highly positive in serum and CSF. Voltage-gated potassium channel complex antibodies, glutamic acid decarboxylase antibodies and a panel of onconeuronal antibodies were negative. Plasma exchange was commenced on day 12; he received 15 single volume cycles without complications. On day 15, CSF demonstrated 2 white cells/mm^3, protein 59 mg/dL, glucose 45 mg/dL,

PCR for HSV type 1 was negative and anti-NMDAR antibodies were negative. Anti-NMDAR serum antibodies were still present on day 15 at low levels but were negative at day 33.

Given the severity of his encephalitis, he took a 6-week tapering course of oral prednisolone, infusions of cyclophosphamide every month for 6 months (cumulative dose of 2250 mg/m^2) and 2 rituximab cycles, given 6 months apart (375 mg/m^2 per day given on 2 consecutive days, 2 weeks apart). A repeat MRI 8 months after presentation revealed atrophy of the right medial temporal lobe with bilateral cerebral parenchymal volume loss and postbiopsy subdural effusions (see Figure D, Supplemental Digital Content 1, http://links.lww.com/INF/C340). He was discharged after 4 months of neurorehabilitation having made a good recovery. At last follow-up, 24 months after presentation, he had no residual physical neurological disability. Formal neuropsychology has not been performed due to his young age but the parents report that his understanding and expression of speech, sleep and behavior are normal. Anti-NMDAR antibodies in serum remain negative.

DISCUSSION

There is increasing evidence of an association between HSV type 1 encephalitis and the presence of anti-NMDAR antibodies. To date, 33 cases of HSV type 1 encephalitis in adults (n = 18) and children (n = 15) have been reported in close temporal relationship to the identification of anti-NMDAR antibodies in the serum and/or CSF[2–9] (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/C341).

Several small case series and case reports have identified patients, primarily children, with clinical relapse following proven HSV type 1 encephalitis. These relapses were associated with development of movement disorders, not seen during the HSV encephalitis, and anti-NMDAR antibodies in the serum, and in CSF when available.[2–7,9] In 7 cases, sera and CSF from the original HSV encephalitis period were shown to be negative for anti-NMDAR antibodies, demonstrating that, in these cases at least, anti-NMDAR antibodies developed only during relapse.[2,4–6]

In another retrospective study, stored acute serum from a cohort of 44 adults diagnosed with HSV type 1 encephalitis (CSF PCR positive) were tested for anti-NMDAR antibodies.[8] None had clinical features suggestive of anti-NMDAR encephalitis, but 13 had anti-NMDAR antibodies with a mixture of IgA, IgM and IgG classes. All the anti-NMDAR antibody positive patients had extensive temporal lobe hyperintensity on MRI imaging in keeping with the typical appearances of HSV type 1 encephalitis, and additional imaging features were not reported.

Our patient differs from those in previous reports as he did not have features of severe clinical HSV type 1 encephalitis during his initial illness, 4 weeks before presentation. Also, the CSF PCR for HSV type 1 was negative throughout the time of hospitalization, although no lumbar puncture was undertaken during his initial mild encephalopathy. Nevertheless, PCR of the parenchyma of the temporal lobe lesion was positive for HSV type 1, 25 days from onset of symptoms and there was histological evidence of infection within some of the neurons. Given his age, it seems likely that this was a primary infection, possibly occurring during his earlier mild encephalopathy.

There are several case series reporting positive HSV type 1 PCR on tissue from patients with epilepsy who had undergone temporal lobectomy,[10] as well as from patients with Rasmussen's encephalitis[11] who did not have clinical HSV type 1 encephalitis. The significance of these findings is not clear, but they may represent reactivation of latent virus in the setting of seizures and neuronal injury.

The temporal lobe changes seen on the MRI in our patient, including hippocampal volume loss, are also consistent with a previous HSV infection with residual damage. MRI findings in anti-NMDAR encephalitis are usually less florid (or normal) and include abnormalities in the medial temporal lobes or less frequently in the thalamus or occipital cortex.[12] Therefore, the evidence of bilateral medial temporal lobe involvement in addition to right-sided diffuse temporal lobe involvement in this case is an important finding that supports features of both HSV type 1 and anti-NMDAR encephalitis. Oedema and T2 hyperintensity on MRI can occur after status epilepticus, with atrophy in severe cases.[13] However, our patient had no clinical or neurophysiologic evidence of seizure activity.

Our patient had a mild and brief encephalopathy 4 weeks before presentation; we therefore hypothesize that he had mild HSV type 1 encephalitis at this point but by admission he was displaying a secondary anti-NMDAR encephalitis syndrome. The clinical features of HSV type 1 infection may have been less severe because the nondominant temporal lobe was most affected. The response to immunotherapy observed and the correlation between the fall in anti-NMDAR antibody titers and clinical improvement suggest that the pathological process at the time of presentation was primarily antibody mediated rather than as a result of viral infection.

The mechanism by which viral infection leads to the production of autoantibodies is not clear. Inflammation and viral neuronal lysis caused by HSV type 1 infection may result in the exposure of neuronal surface proteins, leading to antibody production directed against antigens to which tolerance is normally preserved.[2] Alternatively, a process of molecular mimicry between virus-associated antigens and the NMDA receptor may occur.[8] However, the existence of other infections co-incident with anti-NMDAR encephalitis argues against specific molecular mimicry involving HSV type 1. For example, adenovirus was identified in the CSF of 1 child, and there is 1 case report of anti-NMDAR encephalitis occurring after varicella zoster virus encephalitis.[9,14] Further studies are needed to characterize the role of anti-NMDAR antibodies in HSV type 1 encephalitis, as well as to establish the prevalence of active infection with HSV type 1 or other viruses in patients with anti-NMDAR encephalitis.

Our findings suggest that some patients with anti-NMDAR encephalitis may have had a preceding subtle HSV type 1 infection which may be the precipitant for an autoimmune process, and that more cases of anti-NMDAR encephalitis may be associated with HSV type 1 than currently recognized using CSF PCR only.

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ELISPOT IGRA WITH PURIFIED PROTEIN DERIVATIVE STIMULATION FOR DIAGNOSING NONTUBERCULOUS MYCOBACTERIAL CERVICAL LYMPADENITIS

Antti Kontturi, MD, Tamara Tuuminen, MD, PhD,†‡ Riitta Karttunen, MD, PhD, ‡§ and Eeva Salo, MD, PhD**

Abstract: Childhood cervical lymphadenitis caused by nontuberculous mycobacteria is a diagnostic challenge for the clinician. We present a new promising diagnostic method for childhood nontuberculous mycobacterial lymphadenitis. The modified T-SPOT.TB test with purified protein derivative as an additional antigen is noninvasive with estimated sensitivity and specificity of 1.00 and 0.81, respectively.

Key Words: nontuberculous mycobacterial infection, childhood cervical lymphadenitis, NTM

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From the *Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki; †Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB), Mikkeli District Laboratory, Mikkeli; ‡Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki; and §Laboratory Division (HUSLAB), Helsinki University Central Hospital, Helsinki, Finland.
The authors have no funding or conflicts of interest to disclose.
Address for correspondence: Antti Kontturi, MD, Children's Hospital, University of Helsinki, PB 281, 00029 HUS, Helsinki, Finland. E-mail: antti.kontturi@helsinki.fi; Eeva Salo, MD, PhD, Children's Hospital, University of Helsinki, PB 281, 00029 HUS, Helsinki, Finland. E-mail: eeva.salo@hus.fi.

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Nontuberculous mycobacterial (NTM) infection is an important cause of cervical lymphadenitis in children without Calmette–Guérin bacillus (BCG) vaccination.[1] NTM cervical lymphadenitis usually occurs between 1 and 5 years of age.[1–3] The typical clinical presentation is an otherwise healthy child with unilateral nontender cervical mass.[2,3] The differential diagnoses range in urgency from normal reactive lymph nodes to *Mycobacterium tuberculosis* (MTB) infection and malignancies.[3,4] Eventually, a classic scrofula develops with skin discoloration, thinning and a draining fistula.[2,3] Surgery is considered the primary treatment of choice.[2–4] Delayed diagnosis increases the risk of nerve damage and poor cosmetic outcome.[2,4] Currently, there is no reliable rapid and noninvasive diagnostic method available. Childhood NTM cervical lymphadenitis remains a diagnostic challenge.

The commercial T-SPOT.TB (ELISPOT, Oxford Immunotec Ltd, Oxfordshire, United Kingdom) test is intended to detect patients sensitized to MTB.[5] We have previously reported on modifications applied to this test, including an additional purified protein derivative (PPD; Statens Serum Institut, Copenhagen, Denmark) antigen mixture stimulation of peripheral blood mononuclear cells (PBMCs).[5] The PPD mixture contains dozens of mycobacterial antigens that cross-react with the BCG strains, MTB and many NTM species.[3,6]

During the diagnostic work-up of non-BCG–vaccinated children with suspected NTM cervical lymphadenitis, we observed high reactivity to the PPD antigens without reactivity to MTB specific antigens. This observation encouraged us to evaluate the potential of the modified T-SPOT.TB test for diagnosing NTM cervical lymphadenitis in children.

MATERIALS AND METHODS

The study was conducted according to the guidelines set by the Institutional Review Board of Helsinki University Central Hospital. On review of the hospital records, we found 21 children who had been diagnosed with NTM cervical lymphadenitis in the Hospital District of Helsinki and Uusimaa (HUS) in Finland between March 2009 and January 2012. The patient records and laboratory results of these children were reviewed retrospectively. All children were non-BCG–vaccinated and presented with unilateral nontender cervical mass, either healing after excision or progressing to drainage lasting up to several months. Seventeen had been tested with the modified T-SPOT.TB method. In 10 of these cases, the diagnosis had been confirmed by a positive bacteriological isolation of NTM. These culture-confirmed NTM cervical lymphadenitis cases that had been tested with the modified T-SPOT.TB method formed the disease group (n = 10, 5 male and 5 female). None of the enrolled cases were immunocompromised.

Specimens for conventional cultures (n = 10) had been collected from affected lymph nodes through a biopsy, a fine-needle aspiration or a draining fistula. Mycobacterial species had been identified with GenoType Mycobacterium CM assay (Hain Lifescience, Nehren, Germany). The bacterial isolations were *Mycobacterium avium* (8/10) and *Mycobacterium malmoense* (2/10). The age range in the disease group was 15–38 months with a median age of 31 months. All were born in Finland to Finnish parents. The duration of the lymphadenitis from the first parental observation to the test date ranged from 1 to 8 months with a median of 2 months.

As a control group, we wanted healthy nonsymptomatic non-BCG–vaccinated children tested with the same method. We reviewed the hospital records of all children younger than 5 years tested in HUS in 2009 and 2010. During that period, a total of 99 children had been tested, including 49 BCG-vaccinated, 18 with unclear BCG status and 32 non-BCG–vaccinated. From the 32 non-BCG–vaccinated we excluded those diagnosed with medical condition (n = 11, eg, rheumatoid arthritis, leukemia or pyogenic, mycobacterial or viral infection). The remaining healthy non-BCG–vaccinated children formed the control group (n = 21, 8 boys and 13 girls).