J Neurol (2017) 264:1127–1131
DOI 10.1007/s00415-017-8501-4

ORIGINAL COMMUNICATION

Japanese encephalitis can trigger anti-*N*-methyl-D-aspartate receptor encephalitis

Jiannan Ma[1] · Ting Zhang[2] · Li Jiang[1,3,4,5]

Received: 21 March 2017 / Revised: 26 April 2017 / Accepted: 26 April 2017 / Published online: 3 May 2017

Abstract Japanese encephalitis (JE) is usually a monophasic disease; however, in rare cases, patients with JE may have an early relapse after a partial recovery, giving rise to a biphasic pattern for the disease. In this study, we report three pediatric cases in which post-JE relapse was characterized by movement disorder and/or behavioral problems, and was related to anti-*N*-methyl-D-aspartate receptor (NMDAR) immunoglobulin G (IgG). Serum and cerebrospinal fluid were examined for anti-NMDAR IgG in three patients who had confirmed JE and then developed relapsing symptoms which were similar to those of anti-NMDAR encephalitis. The main symptoms of the two young children were choreoathetosis, irritability, and sleep disorder; while for the teenager, agitation, mutism, rigidity, and sleep disorder were the main symptoms. Samples of cerebrospinal fluid from all patients were positive for anti-NMDAR IgG, and all patients gradually improved with immunotherapy. Testing for NMDAR antibodies is highly recommend in patients with JE, especially those with a relapsing syndrome involving movement disorder and/or behavioral problems, as these patients may benefit from immunotherapy.

Keywords Japanese encephalitis · Anti-NMDAR encephalitis · Movement disorder · Relapsing

✉ Li Jiang
dr_jiangli@163.com

1 Department of Neurology, Children's Hospital of Chongqing Medical University, No. 136 Zhongshan 2nd Road, Yuzhong District, Chongqing 400014, People's Republic of China

2 Department of Rehabilitation, Children's Hospital of Chongqing Medical University, Chongqing 400014, People's Republic of China

3 Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing 400014, People's Republic of China

4 China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing 400014, People's Republic of China

5 Chongqing Key Laboratory of Translational Medical Research in Cognitive Development and Learning and Memory Disorders, Chongqing 400014, People's Republic of China

Introduction

Japanese encephalitis (JE), a mosquito-borne flavivirus disease, is one of the most important causes of viral encephalitis in children in Asia, with potentially devastating consequences, including severe neurodisability and developmental impairment [1]. It is usually a monophasic disease, characterized by fever, headaches, seizures, and altered consciousness; however, in rare cases, patients with JE may have an early relapse after a partial recovery, giving rise to a biphasic pattern for the disease. According to limited reports in the literature, the clinical manifestations of the second phase of JE are different from the first phase, in that mutism, dystonia, peri-oral dyskinesia, and behavioral changes are more conspicuous [2]. We have also observed similar phenomena in our own pediatric clinical practice (unpublished observations).

Interestingly, the symptoms of the second phase of JE are very similar to those of anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis. For anti-NMDAR encephalitis, the common early symptoms are behavioral and speech problems, seizures, and abnormal movements [3]. Furthermore, a handful of predominantly pediatric cases of neurological relapse after herpes simplex virus

(HSV) encephalitis were reported to be associated with NMDAR antibodies in recent studies [4–6].

The evidence above led us to postulate that NMDAR antibodies might play a role in post-JE relapse. In the current study, we report three pediatric cases in which post-JE relapse was characterized by movement disorder and/or behavioral problems, and was related to anti-NMDAR IgG. To our knowledge, investigations of NMDAR antibodies have not been previously reported in similar cases.

Methods

From September to October 2016, three children were readmitted for post-JE relapse to the Children's Hospital of Chongqing Medical University, the largest tertiary medical center for children in south-west China.

A detailed clinical examination, magnetic resonance imaging (MRI) scan of the brain, and a routine cerebrospinal fluid (CSF) examination were conducted. Serum and CSF were examined for anti-NMDAR IgG. Patient medical records from the first phase of JE were retrospectively studied (all patients in this study were treated in our hospital during the first phase).

Using a cell-based assay, we tested for the presence of anti-NMDAR IgG via indirect immunofluorescence on HEK293 cells transfected with homodimers of the NR1a subunit of the NMDAR, according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). Briefly, HEK293 cells were fixed to object plates and incubated with patient specimens (native CSF samples, serum diluted 1:10–1:100), followed by washing and then incubation with fluorescent-labeled anti-human IgG antibody. Antibody binding was assessed by light microscopy (EUROStar III; Euroimmun) and compared to standardized positive and negative controls.

Informed consent was obtained from the parents or legal guardian of each child. The study was approved by the ethics committee of the Children's Hospital of Chongqing Medical University.

Results

The clinical features of the three patients are summarized in Table 1. Patient 1 and patient 3 were young children, whereas patient 2 was 14 years. All patients were from JE epidemic area and were unvaccinated. Initially, they presented with symptoms including high fever, headache, increasing loss of consciousness, and seizures, which are typical clinical manifestations of JE. Examination of CSF showed pleocytosis and raised protein levels in all patients, while JE virus-specific IgM in serum and CSF was positive by IgM-capture ELISA, confirming the diagnosis of JE. Magnetic resonance imaging (MRI) scans showed predominant affection of bilateral thalamus (Fig. 1a, d, g). On supportive treatments, which included cooling, anticonvulsants (if seizures occurred), and decreasing intracranial pressure, all patients gradually recovered and were discharged within 2–3 weeks. Patient 1 had rehabilitation treatment afterwards because of residual motor and cognitive deficits, while patients 2 and 3 went straight home with no sequelae.

However, all three patients developed new symptoms between days 25 and 29 following first presentation. The main symptoms of patients 1 and 3 were choreoathetosis, irritability, and sleep disorder, while for patient 2, they were agitation, mutism, rigidity, and sleep disorder. Follow-up MRI scans approximately 1 month after onset showed larger, more conspicuous lesions with no effects on other parts of the brain (Fig. 1b, e, h). Analyses of CSF showed raised protein levels with normal cell counts. Patient 1 had anti-NMDAR IgG in both serum and CSF, while patients 2 and 3 had anti-NMDAR IgG in CSF only (Fig. 2).

After confirming the diagnosis of anti-NMDAR encephalitis, immunotherapy was conducted using intravenous immunoglobulin (400 mg/kg/day for 5 days) and intravenous methylprednisolone (15–20 mg/kg/day for 5 days), followed by long-term oral administration of prednisone (1.5–2.0 mg/kg/day). All patients gradually improved. Patients 1 and 2 had follow-up MRI scans, which showed partial absorption of earlier lesions (Fig. 1c, f).

Discussion

In this study, we reported findings from three children exhibiting post-JE relapse accompanied by anti-NMDAR IgG in their CSF.

The diagnosis of anti-NMDAR encephalitis was made carefully step-by-step, following diagnostic criteria mainly based on guidelines for diagnosis of anti-NMDAR encephalitis [7]. A probable diagnosis can be made using neurological assessment and conventional tests accessible to most clinicians. A positive result for IgG anti-GluN1 antibody then permits a definite diagnosis, leading to prompt immunotherapy [7].

The patients in our study had symptoms typical of anti-NMDAR encephalitis; the two young children mainly presented with movement disorders, whereas the teenager mainly presented with behavioral problems. These symptoms are in line with those reported in a large observational cohort study of 577 patients with anti-NMDAR encephalitis [8]. We then confirmed the

Table 1 Clinical features of three patients with autoimmune relapsing symptoms after Japanese encephalitis

Patient no.	Age, sex	Japanese encephalitis				Time of relapse	Relapse				Follow-up
		Symptoms	CSF	Time of MRI and location of FLAIR hypersignal	Time and condition at discharge		Symptoms	CSF	Time of MRI and location of FLAIR hypersignal	NMDAR Abs IgG	
1	30 months, M	Fever, seizures, coma	WBC: 13 Prot: 111 JE-IgM: +	Day 7; bilateral thalamus	Day 20, motor and cognitive residual deficits	Day 29	Choreoathetosis, peri-oral dyskinesia, irritability, sleep disorder, seizure	WBC: 6 Prot: 102	Day 31; bilateral thalamus, more conspicuous and expanded than original scan	Serum: + CSF: +	Day 80; reduction of choreoathetosis and peri-oral dyskinesia, improvement of sleep
2	14 years, M	Fever, headache, vomiting, coma	WBC: 29 Prot: 80 JE-IgM: +	Day 6; bilateral thalamus	Day 14, complete recovery	Day 25	Agitation, mutism, rigidity, sleep disorder	WBC: 4 Prot: 83	Day 35; bilateral thalamus, more conspicuous and expanded than original scan	Serum: − CSF: +	Day 68; simple verbal response, emotional instability
3	6 years, F	Fever, headache, vomiting, coma	WBC: 35 Prot: 92 JE-IgM: +	Day 7; bilateral thalamus	Day 15, complete recovery	Day 27	Choreoathetosis, irritability, sleep disorder	WBC: 3 Prot: 78	Day 28, bilateral thalamus, more conspicuous than original scan	Serum: − CSF: +	Day 64; reduction of choreoathetosis, improvement of sleep

M male, *F* female, *CSF* cerebrospinal fluid, *WBC* white blood cells/μl in CSF, *Prot* CSF total protein (mg/dl), *IgM* immunoglobulin M, *MRI* magnetic resonance imaging, *FLAIR* fluid-attenuated inversion recovery, *JE* Japanese encephalitis, *NMDAR* *N*-methyl-D-aspartate receptor, *Abs* antibodies

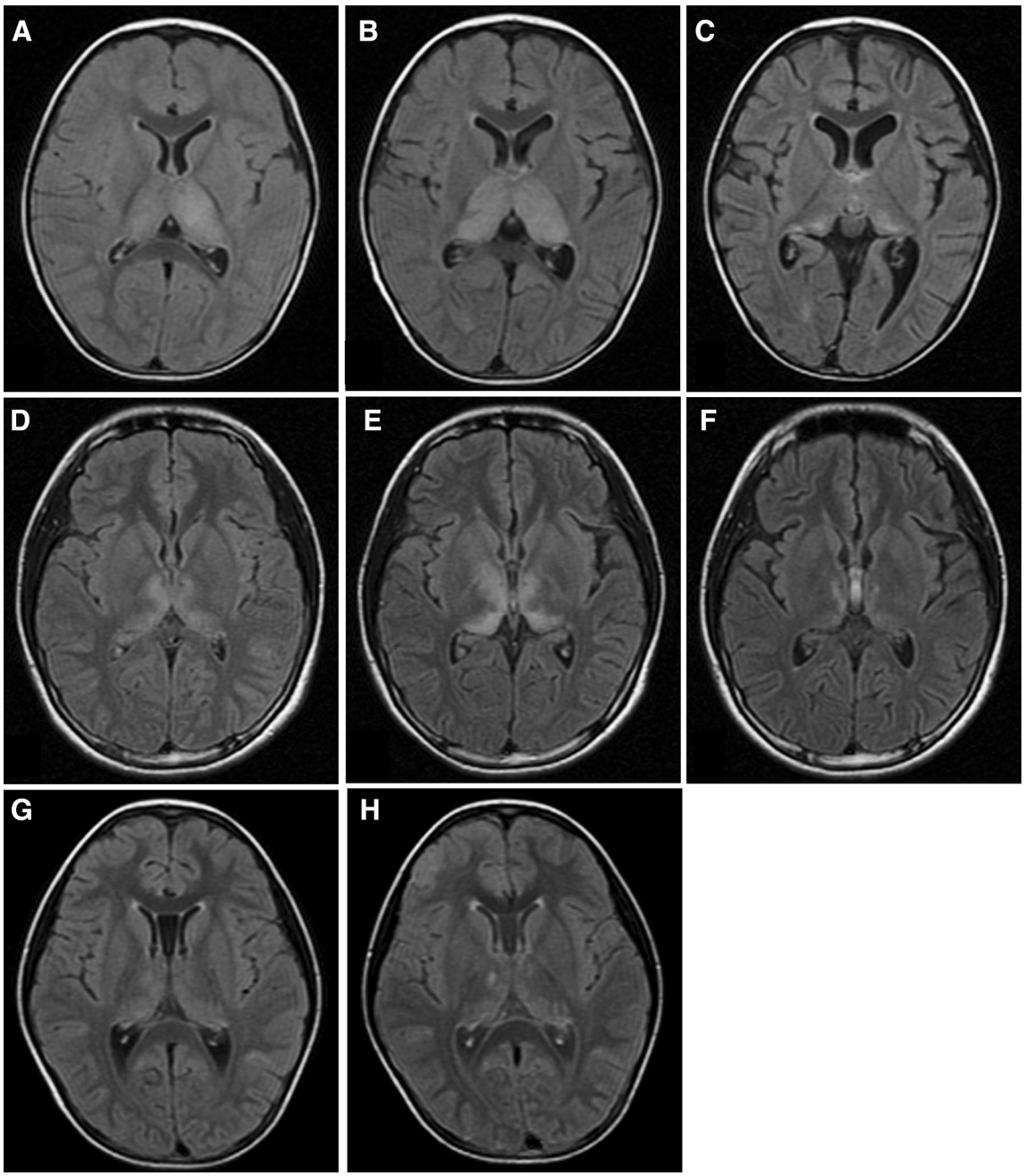

Fig. 1 Axial fluid attenuation inversion recovery (FLAIR) MRI scans of patient 1 (**a**–**c**), patient 2 (**d**–**f**), and patient 3 (**g**, **h**). **a**, **d**, **g** Images during JE on days 6–7 showed a diffuse or patchy hypersignal in bilateral thalamus. **b**, **e**, **h** Follow-up images during post-JE relapse on days 28–35 revealed that the hypersignal was more conspicuous and expanded (**b**, **e**) or more conspicuous but unexpanded (**h**) in bilateral thalamus, compared to the hypersignal from the original image. **c**, **f** Follow-up images on day 80 (**c**) and day 68 (**f**) showed the partial absorption of previously affected regions

diagnoses of anti-NMDAR encephalitis in our patients by antibody testing. All of them responded to immunotherapy, which further confirms the diagnosis of anti-NMDAR encephalitis [9].

Diagnosis of anti-NMDAR encephalitis requires the reasonable exclusion of other related disorders [7]. In our patients, a diagnosis of latent JE reactivation was excluded, based on the following reasons: first, typical symptoms from the first phase of JE seldom appear in the second phase, which imply different pathological physiology between the two phases; second, no new lesions were found in MRI scans during the second phase of illness; and finally, patients improved rather than deteriorated following immunotherapy.

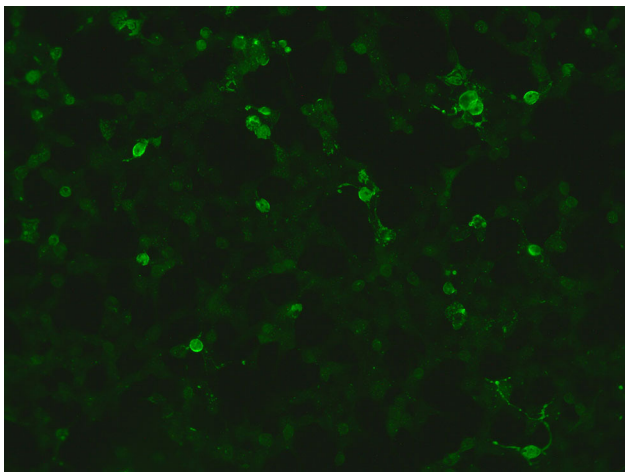

Fig. 2 Positive reaction with transfected HEK293 cells expressing the glutamate receptor (type NMDA; subunit NR1a) after incubation with cerebrospinal fluid of one of our patients followed by anti-immunoglobulin secondary antibody

In fact, Japanese encephalitis is not the first viral encephalitis to be linked with autoimmune encephalitis. It is widely accepted that HSV encephalitis can trigger NMDAR antibodies and potentially other autoimmune responses in the brain, leading to a second phase of immune-mediated encephalopathy [4–6, 10, 11]. The diagnostic guidelines mentioned above specifically state that patients with a history of HSV encephalitis in the previous weeks might have relapsing immune-mediated neurological symptoms (post-HSV encephalitis) [7]. Armangue et al. speculated that the release of antigens by viral neuronal lysis and inflammation may lead to synaptic autoimmunity [4]. Interestingly, they cite supporting evidence including similarities in neurological complications observed over a bimodal clinical course in other forms of viral encephalitis such as JE. Our findings further confirm this speculation.

This study is limited by the scarcity of samples from the first phase of post-JE relapse patients and the convalescence of monophasic JE patients. Therefore, we cannot determine whether JE normally triggers brain autoimmunity in the early stage of the illness or right before the relapsing stage. Furthermore, we were unable to test for JE virus RNA in post-JE relapse patients because of technical limitations and, therefore, cannot completely exclude the possibility of a reactivation of latent JE infection. As such, the present findings need further evaluation in future studies. However, we highly recommend testing for NMDAR antibodies in patients with JE, especially those with a relapsing syndrome involving movement disorder and/or behavioral problems, as these patients may benefit from immunotherapy.

Acknowledgements We would like to express our sincere gratitude to all the patients and their families who participated in this study. We also thank all the medical and allied health staff members who cared for the children in this study.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standards Informed consent was obtained from the parents or legal guardian of each child. The study was approved by the ethics committee of the Children's Hospital of Chongqing Medical University.

References

1. Vaughn DW, Hoke CH Jr (1992) The epidemiology of Japanese encephalitis: prospects for prevention. Epidemiol Rev 14:197–221
2. Pradhan S, Gupta RK, Singh MB, Mathur A (2001) Biphasic illness pattern due to early relapse in Japanese-B virus encephalitis. J Neurol Sci 183:13–18
3. Florance NR, Davis RL, Lam C et al (2009) Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. Ann Neurol 66:11–18
4. Armangue T, Leypoldt F, Malaga I et al (2014) Herpes simplex virus encephalitis is a trigger of brain autoimmunity. Ann Neurol 75:317–323
5. Hacohen Y, Deiva K, Pettingill P et al (2014) N-methyl-D-aspartate receptor antibodies in post-herpes simplex virus encephalitis neurological relapse. Mov Disord 29:90–96
6. Bamford A, Crowe BH, Hacohen Y et al (2015) Pediatric herpes simplex virus encephalitis complicated by *N*-methyl-D-aspartate receptor antibody encephalitis. J Pediatric Infect Dis Soc 4:e17–e21
7. Graus F, Titulaer MJ, Balu R et al (2016) A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol 15:391–404
8. Titulaer MJ, McCracken L, Gabilondo I et al (2013) Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol 12:157–165
9. Suleiman J, Brilot F, Lang B, Vincent A, Dale RC (2013) Autoimmune epilepsy in children: case series and proposed guidelines for identification. Epilepsia 54:1036–1045
10. Hoftberger R (2015) Neuroimmunology: an expanding frontier in autoimmunity. Front Immunol 6:206
11. Titulaer MJ, Leypoldt F, Dalmau J (2014) Antibodies to *N*-methyl-D-aspartate and other synaptic receptors in choreoathetosis and relapsing symptoms post-herpes virus encephalitis. Mov Disord 29:3–6