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Case Report

Cytokine/chemokine elevation during the transition phase from HSV encephalitis to autoimmune anti-NMDA receptor encephalitis

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Abstract

A 3-year-old girl suffered from anti-N-methyl-D-aspartate (anti-NMDA) receptor encephalitis after resolution of herpes simplex virus encephalitis (HSE). Methylprednisolone pulse and immunoglobulin therapies showed little effect, but the patient completely recovered after six courses of monthly cyclophosphamide pulse therapy and successive maintenance on mycophenolate mofetil for one year. Anti-NMDA receptor antibody in the cerebrospinal fluid (CSF) was minimally detected during the prodromal febrile period and then was seen to be markedly elevated at the onset of second encephalopathy phase. CSF interleukin (IL)-6, and 10, tumor necrosis factor-α, interferon gamma, C-X-C motif ligands (CXCL)10 and 13, chemokine ligand 2, and migration inhibitory factor showed a second peak during the prodromal period and were reduced at the onset of anti-NMDA receptor encephalitis. These suggest the presence of cytokine/chemokine phase between the initial HSE and the secondary autoimmune encephalitis phases. Treatment strategy during the early stage of this entity should be further explored.

Keywords: Herpes simplex virus; Anti-NMDA receptor antibody; Cyclophosphamide; CXCL13; Monocyte/microglia

1. Introduction

Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis was first recognized as a subgroup of non-herpetic temporal lobe encephalitis in young women, accompanying ovarian teratoma. Apart from this condition caused by autoimmune response against NMDAR-expressing tumor cells, anti-NMDAR encephalitis in the absence of tumors have also been identified in children, some of whom showed evidence of preceding infections with mycoplasma, influenza, and herpes zoster viruses [1].

More recently, anti-NMDAR encephalitis has been proven to emerge after initial resolution of herpes simplex virus encephalitis (HSE) as a relapsing course of neurological illness [2]. Production of anti-NMDAR antibody in the cerebrospinal fluid (CSF) has been hypothesized as caused by the antigen exposure to the immune system by viral neuronal lysis. Differential elevation of CSF cytokines/chemokines during the HSE phase and post-HSE anti-NMDAR encephalitis phase was identified in a single case supporting the provocation of secondary inflammatory process [3]. We herein report another case of post-HSE anti-NMDAR encephalitis, where we could find elevation of a particular

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set of CSF cytokines/chemokines during the prodromal phase before emergence of second encephalopathy phase.

2. Case report

A previously healthy 3-year-old female developed a prolonged generalized convulsion after 3 days of high-grade fever and headache, and was admitted to our hospital (day 1). After termination of the convulsion, she could recognize her parents, but she showed partially impaired alertness and verbal fluency. CSF analysis revealed pleocytosis of 310/mm^3 and positive herpes simplex virus (HSV) polymerase chain reaction (PCR). At this stage, serum and CSF NMDAR antibodies were negative. Magnetic resonance imaging (MRI) revealed high signal in the left anterior, medial temporal regions (Fig. 1A and B). She was treated with intravenous acyclovir (45 mg/kg/day) and successively with addition of intravenous immunoglobulins (IVIg; 2 g/kg) and Ara-A (15 mg/kg/day). The pyrexia resolved by day 7, and the patient behaved normally on day 8. Pyrexia re-emerged from day 12 and gradually aggravated until day 20. CSF on day 10 was negative for HSV-PCR, but pleocytosis persisted. Despite treatment with methylprednisolone pulse (MP) therapy with 30 mg/kg/day for 3 days and another IVIg therapy, the patient became vacant on day 28 and developed progressive irritability, movement disorder (chorea, dystonia, and stereotypic movements), and autonomic disturbances, such as tachycardia, low-grade fever, and insomnia on day 30 and thereafter (Fig. 2). Consecutive MRI at this period revealed propagation of the high signal to the anterior-lateral areas adjacent to the initial HSV lesion, involving both gray and white matters (Fig. 1C–E). Repeated IVIg and MP therapies showed little effect, and the patient lost total social contact. After plasma exchange on day 47, her alertness and movement disorder gradually improved. However, she was still hyporeactive and developed progressive irritability again.

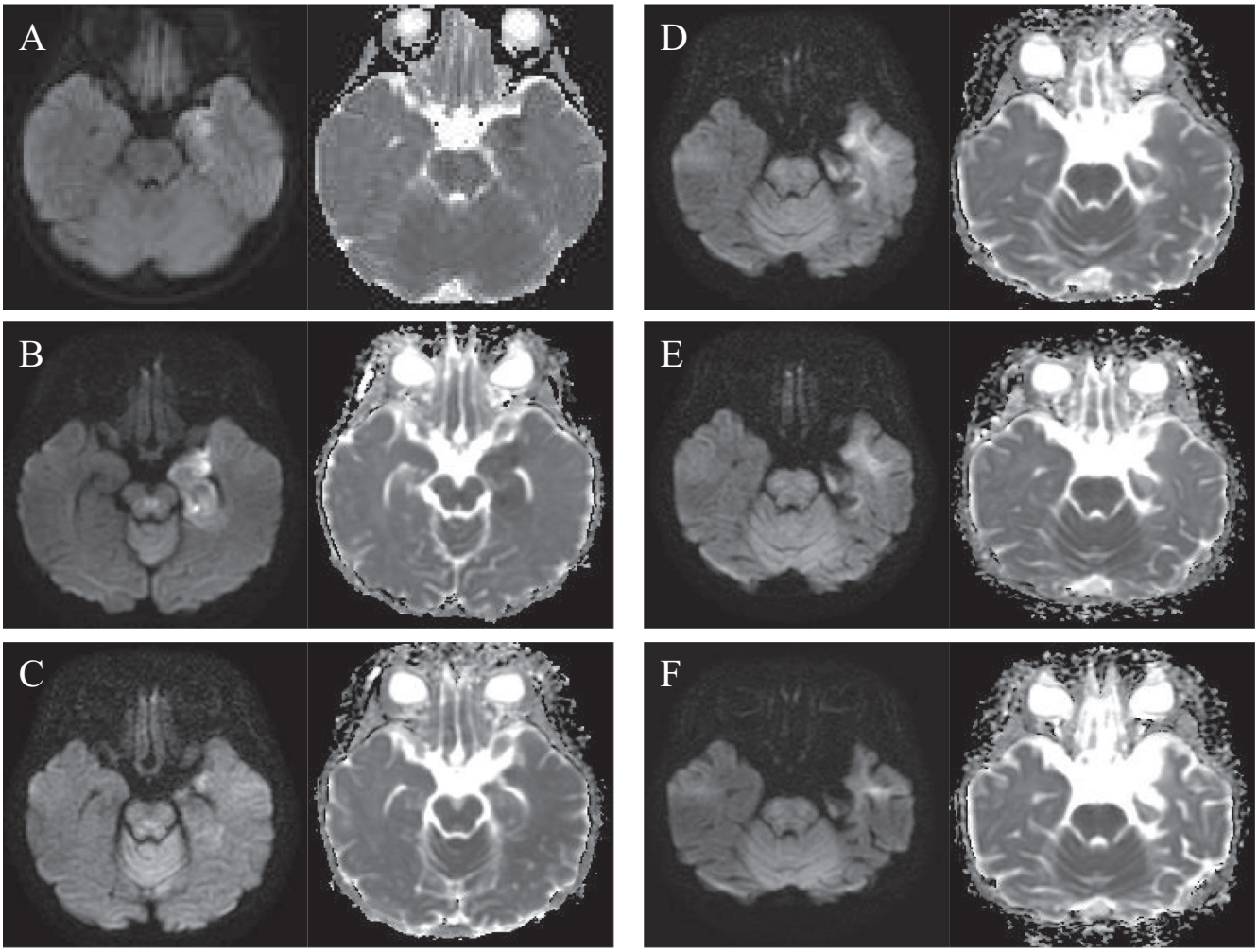
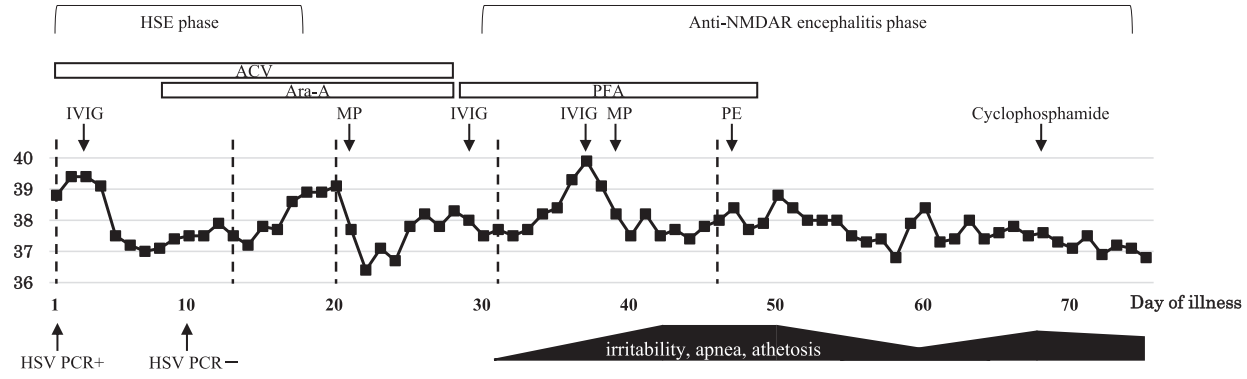


Fig. 1. Magnetic resonance imaging on days 2 (A), 4 (B), 18 (C), 28 (D), 31 (E), and 46 (F) of admission. Diffusion-weighted image and apparent diffusion coefficient map are shown in each panel on the left and right sides, respectively.



CRP (ug/mL)	0.02		0.06	3.62	0.20		0.04	0.06	0.41	2.21	0.75	1.10	<0.01
WBC (/μl)	8500		3700	5900	6100		9300	4200	7600	3200	15000	7400	7800
CSF cell count	331		131	52	213		67	30			9		6
CSF protein	33		39	31	55		35	41			25		40

Fig. 2. Clinical course of the patient. With a concern of possible viral reactivation, ACV and Ara-A was replaced by intravenous foscarnet (PFA, 180 mg/kg/day) on day 28, which was then terminated after confirmation of negative results in high-sensitivity HSV-PCR analysis. ACV, acyclovir; Ara-A, vidarabine; CSF, cerebrospinal fluid; HSE, herpes simplex virus encephalitis; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin; MP, methylprednisolone pulse therapy; NMDAR, anti-*N*-methyl-$_D$-aspartate receptor; PE, plasmapheresis; PFA, foscarnet.

Then, cell-based analysis of CSF samples on days 31 and 46 yielded positive results for anti-NMDAR antibody (Fig. 3). Cyclophosphamide (CYC) pulse therapy was started on day 68 (monthly doses of 500 mg/m^2 for 6 months), followed by maintenance with mycophenolate mofetil (MMF; 600 mg/m^2/day) for 1 year. Verbal communication appeared after the second CYC pulse, and the patient achieved full recovery by 6 years of age, 6 months after termination of MMF. Her intelligence quotient was assessed as 90 on Wechsler Intelligence Scale for Children-IV.

3. Evolution of CSF cytokines/chemokines and anti-NMDAR antibody during the biphasic illness

Cytokines [interferon gamma (IFNγ), interleukin (IL)-1β, IL-6, IL-10, migration inhibitory factor (MIF), and tumor necrosis factor alpha (TNFα)] and chemokines [chemokine ligand 2, CCL2; C-X-C motif ligand (CXCL)8, CXCL10, CXCL13, and Gro-α] in the CSF were measured by bead-based multiplex assay using Bio-plex Pro Human Chemokine Panel (Bio-Rad, Richmond, CA) according to the manufacturer's protocol. Autoantibody to NMDA receptor was quantified by flow cytometry. Briefly, live HEK293 cells transfected with NR1 and NR2B subunits of human NMDA receptor, as well as untransfected HEK 293 cells, were reacted with diluted (1:10) CSF and then stained with Alexa fluor 647-conjugated anti-human IgG secondary antibody (Jackson Immunoresearch, West Grove, PA). Antibody titers were semi-quantified and were shown as the difference in the mean fluorescence intensity between NMDA receptor-transfected and untransfected HEK293 cells (= ΔMFI).

During the initial phase of HSE, various cytokines/chemokines were elevated in the CSF, including those recruiting neutrophils (CXCL8/IL-8 and Gro-α), activating monocyte cell lineage (MIF, CCL2), T-helper related cytokines/chemokines (CXCL10, IFNγ, TNFα, and IL-6), broad inflammatory conductor IL-1β, as well as inhibitory cytokine IL-10 (Fig. 3). These cytokines/chemokines were all observed to be reduced on day 13, and IL-1β and Gro-α remained negative thereafter. Others and a B-cell chemokine CXCL13 were elevated on day 19 at the onset of recurrent fever before emergence of relapsing neurological symptoms of anti-NMDAR encephalitis. Then, at the onset of neurological relapse on day 31, anti-NMDAR antibody was first elevated in the CSF. Cytokines/chemokines were reduced on day 31, but were still positive for MIF, CCL2, CXCL10, and TNFα. During immunotherapy with MP and CYC, anti-NMDAR antibody was gradually reduced and most of the cytokines/chemokines remained negative. In contrast, MIF, CCL2, and CXCL10 persisted in the CSF, as did CXCL8 at low levels until day 256. Serum anti-NMDAR antibody was negative on day 1, but was positive on day 46.

4. Discussion

Initial broad elevation of CSF cytokines/chemokines during the HSE phase and a second peak of T-cell and B-cell during the prodromal phase was consistent with the previous report on post-HSE anti-NMDAR

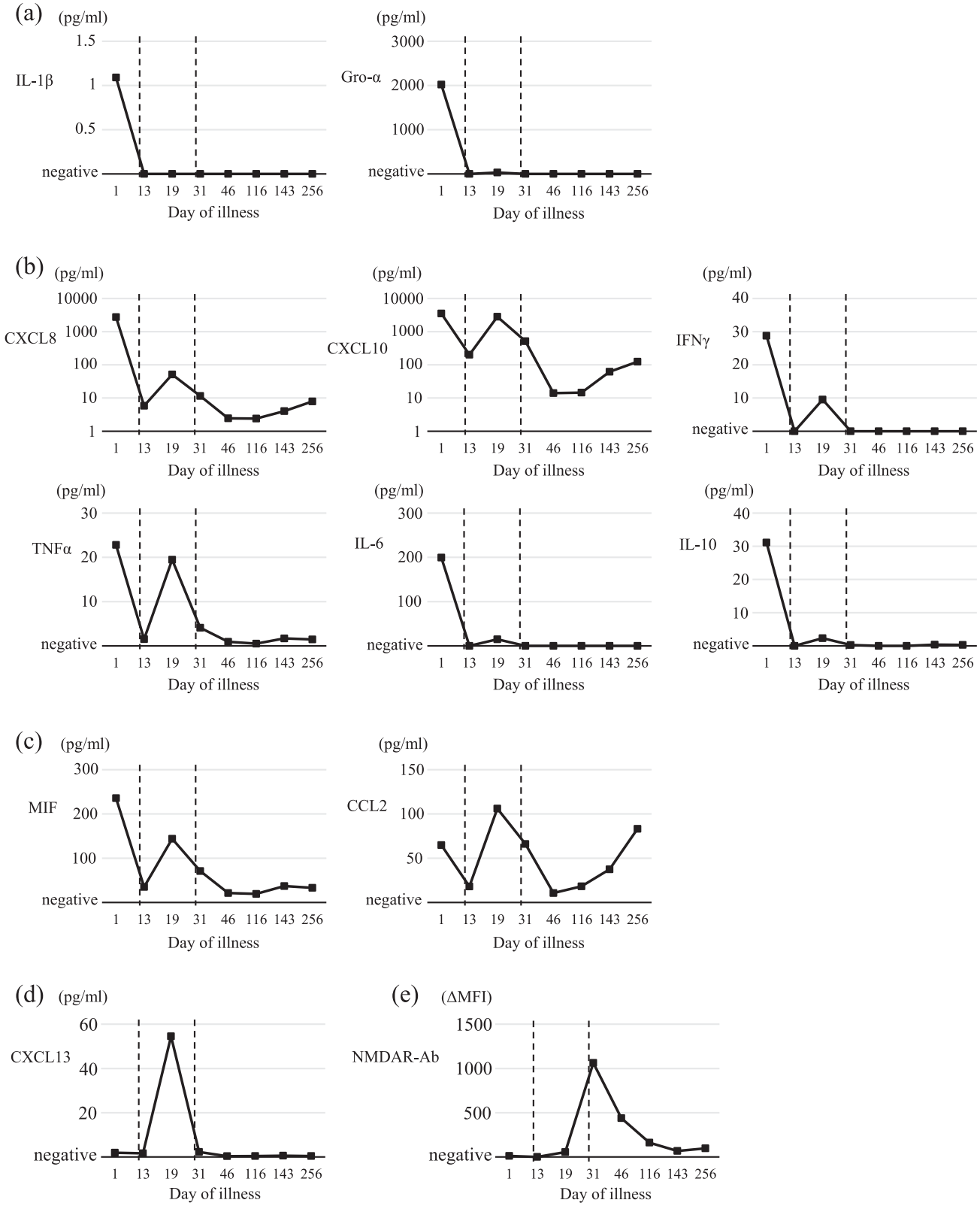


Fig. 3. Evolution of cerebrospinal fluid (CSF) cytokines/chemokines and anti-NMDA receptor antibody during the biphasic course of herpes simplex virus encephalitis (HSE) and post-HSE anti-NMDA receptor encephalitis. Cyto-/chemokine and anti-NMDA receptor antibody were measured for the CSF samples on day 1 (the day of admission and onset of initial HSE), day 19 (febrile period after resolution of HSE), day 31 (onset of neurological relapse), day 46 (after second MP therapy), day 116 (after two courses of CYC pulse therapy), and days 143 and 256 (after six courses of CYC pulse therapy, with full intellectual recovery and residual tendency of compulsive or inattentive behaviors).

encephalitis [3]. In addition, we could find, for the first time, a minimal production of anti-NMDAR antibody at the prodromal phase before the severe secondary encephalopathy phase. Persistent elevation of CSF monocyte/macrophage cytokines/chemokines (MIF and CCL2) and IL-1β during the recovery phase for several months, along with residual anti-NMDAR antibody, was also confirmed.

In anti-NMDAR encephalitis, either post-HSE or not, approximately half (30–70%) of the patients respond well to first-line treatment with steroids, IVIg, and plasmapheresis [1,4–7]. However, IVIg and MP during the prodromal phase with elevation of CSF cytokines/chemokines could not prevent the emergence of anti-NMDAR encephalitis. As a potential biomarker for prediction of prognosis in anti-NMDAR encephalitis, CSF CXCL13 was higher in patients with prodromal fever or headaches and with limited response to therapy, and its levels were correlated with intrathecal synthesis of NMDAR antibodies [8]. In the present case and another [3], both refractory to the first-line treatment and significantly benefited from second-line treatment with cyclophosphamide or rituximab, CXCL13 was elevated to 50 pg/ml or higher during the prodromal period. This was well beyond the overlap range with control samples [8], which support the predictive value of this chemokine for anti-NMDAR encephalitis before the emergence of secondary encephalopathy phase. In refractory anti-NMDAR encephalitis, the number of plasmablasts was increased, and the strategies for targeting antibody-producing cells may be effective [9]. Thus, first-line immunotherapy should be initiated during the prodromal phase with confirmation of elevated CSF CXCL13 and/or other chemokines/cytokines, and second-line immunotherapy should be promptly considered in the emergence of second encephalopathy despite under first-line therapies during the prodromal phase.

MIF and CCL2 persisted in the CSF, as did CXCL10 until day 256 when the patient showed residual behavioral problems. These prompted us to initiate the maintenance therapy with MMF, which suppresses the cytokine production from helper T lymphocytes and monocytes, as well as the antibody formation from B-lymphocytes [10], and listed in the second-line immunotherapy for post-HSE anti-NMDAR encephalitis [7]. Indeed, brain biopsy from a patient with anti-NMDAR encephalitis showed abundant CXCL13 expression in the perivascular activated monocytes/macrophages and parenchymal microglia [8]. As persistent CXCL13 action can lead to long-lasting antibody production, monocyte lineage cells may play a pivotal role during the chronic phase up to years after onset in post-HSE anti-NMDAR encephalitis.

In these points, our results would contribute to further exploration of the autoimmune mechanism and treatment strategy for this entity.

Acknowledgement

The authors claim no conflict of interest.

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