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Raised cerebrospinal fluid BAFF and APRIL levels in anti-*N*-methyl-D-aspartate receptor encephalitis: Correlation with clinical outcome

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ABSTRACT

In this study, we aimed to assess the levels of B cell activating factor from the tumor necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL) in cerebrospinal fluid (CSF) of patients with anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis and determine their correlation with clinical outcome. BAFF and APRIL concentrations in CSF and serum from 40 patients with anti-NMDAR encephalitis and 20 controls were measured by enzyme-linked immunosorbent assay (ELISA). Compared with controls, the levels of both BAFF and APRIL in CSF were significantly increased in patients with anti-NMDAR encephalitis ($p < 0.001$ and $p < 0.001$). Patients with unfavorable outcome had higher levels of BAFF and APRIL in CSF than those who had favorable outcome ($p < 0.05$ and $p < 0.05$). BAFF and APRIL levels in CSF were elevated and associated with clinical outcome in patients with anti-NMDAR encephalitis, indicating that they may be valuable biomarkers to this disease.

1. Introduction

Anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is a newly recognized autoimmune disease of the central nervous system (CNS) (Dalmau et al., 2011). This disorder mainly affects young adults and children. Clinical manifestations of the disease often present with acute or subacute psychiatric and neurological symptoms, including abnormal behaviors, speech dysfunction, memory deficits, seizures, movement disorder, autonomic dysfunction, central hypoventilation and even declined consciousness (Graus et al., 2016).

Anti-NMDAR encephalitis is characterized by abnormal production of autoantibodies to the GluN1 subunit of NMDAR (Dalmau et al., 2011). These autoantibodies have direct pathogenic effects by inducing crosslink and internalization of NMDAR (Hughes et al., 2010; Planaguma et al., 2015). It has been found that the concentrations of autoantibodies in cerebrospinal fluid (CSF) are higher than that in sera in patients with integrated blood-brain barrier (BBB) (Dalmau et al., 2008), indicating intrathecal synthesis of autoantibodies. Furthermore, infiltration of plasma cells and plasmablasts in patients' brain and expansions of B cells in CSF have also been observed (Dale et al., 2013; Martinez-Hernandez et al., 2011). These findings suggest that B cells are recruited and activated in CNS of patients with anti-NMDAR encephalitis.

The B cell activating factor from the tumor necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL) are two critical cytokines for the survival and maturation of B cells and the production of antibodies (Bossen and Schneider, 2006; Vincent et al., 2013). They share two receptors: B-cell maturation antigen (BCMA) and transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI). In addition, BAFF can bind to the BAFF receptor (BAFF-R) specifically (Bossen and Schneider, 2006). These three receptors are mainly expressed in the membrane of B cells. After BAFF and APRIL binding to their receptors, the survival and growth signals could be transmitted to B cells (Bossen and Schneider, 2006; Castigli et al., 2005). The levels of BAFF and APRIL in CSF have been shown to be increased in patients with various neurological autoimmune disorders, such as neuromyelitis optica, multiple sclerosis, neuropsychiatric systemic lupus erythematosus and opsoclonus-myoclonus syndrome, suggesting that both BAFF

Abbreviations: NMDAR, *N*-methyl-D-aspartate receptor; CNS, central nervous system; CSF, cerebrospinal fluid; BBB, blood-brain barrier; BAFF, B cell activating factor from the tumor necrosis factor family; APRIL, a proliferation-inducing ligand; BCMA, B-cell maturation antigen; TACI, transmembrane activator and calcium-modulating cyclophilin ligand interactor; CXCL13, C-X-C motif ligand 13; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; mRS, modified Rankin Scale; ELISA, enzyme-linked immunosorbent assay.

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and APRIL participate in the autoimmune process in CNS (George-Chandy et al., 2008; Kothur et al., 2016a; Pranzatelli et al., 2013; Quan et al., 2013; Wang et al., 2012).

Since B cells and autoantibodies have important role in the pathogenesis of anti-NMDAR encephalitis, it is reasonable to speculate that the levels of B cell-related cytokines and chemokines in CSF are elevated in patients with anti-NMDAR encephalitis. Indeed, recent studies have shown that C-X-C motif ligand 13 (CXCL13), a major chemokine that attracts B cells, is elevated in CSF of patients with anti-NMDAR encephalitis (Byun et al., 2016; Kothur et al., 2016b; Leypoldt et al., 2015; Liba et al., 2016). However, previous studies suggested that the levels of BAFF and APRIL were not elevated in CSF of patients with anti-NMDAR encephalitis, which were inconsistent with the change of CXCL13 (Kimura et al., 2015; Kothur et al., 2016b; Liba et al., 2016). Whether BAFF and APRIL are increased in CSF of patients with anti-NMDAR encephalitis remains to be studied.

In this study, we aimed to explore the potential biomarkers of anti-NMDAR encephalitis by detecting the changes of BAFF and APRIL levels in CSF and further investigate their correlation with clinical outcome in patients with anti-NMDAR encephalitis.

2. Patients and methods

2.1. Patients

Forty patients with anti-NMDAR encephalitis were recruited from January 1, 2009, to December 31, 2015, at Huashan Hospital, Fudan University. The patients' diagnosis were all confirmed by clinical manifestations and detection of anti-NMDAR antibodies in the CSF samples using cell-based assay according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). It has been proposed that anti-NMDAR encephalitis could be divided into 4 stages: prodrome and initial psychiatric symptoms stage, neurologic complications stage, recovery stage, late-phase cognitive and behavioral sequelae stage (Kayser and Dalmau, 2011). To simplify this classification, we combined the first two stages as acute stage, while the other two stages as recovery stage. Of all the 40 patients, 21 and 13 patients' CSF were collected in acute stage and recovery stage respectively. Six patients had both CSF samples at acute stage and recovery stage. Eight patients had clinical relapses, and 4 of them had CSF samples at acute stage of both first and relapse courses. Occult tumor screening was performed in all patients using at least one of the following techniques: computed tomography (CT), magnetic resonance imaging (MRI), B-mode ultrasound and positron emission tomography (PET). Patients were followed up at least 3 months, and their clinical outcome were assessed by using modified Rankin Scale (mRS).

CSF and serum of 20 randomly selected patients with noninflammatory neurological diseases: headache ($n = 9$), peripheral nerve injury ($n = 2$), painful ophthalmoplegia ($n = 2$), vertigo ($n = 1$), hereditary cerebellar ataxia ($n = 1$), congenital muscular dystrophy ($n = 1$), spinal cavernous hemangioma ($n = 1$), delayed radiation myelopathy ($n = 1$), hypophysoma ($n = 1$) and lumbocrural pain ($n = 1$) served as controls. All control CSF samples were normal with routine CSF analyses.

Informed consent was obtained from all patients or their families. This study was approved by the Ethical Committee of Huashan Hospital, Fudan University.

2.2. Detection of BAFF and APRIL in CSF and serum

All collected CSF and serum samples were stored at −80 °C until detection. The concentrations of BAFF and APRIL were measured using pre-coated enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions (Quantikine; R&D Systems, Minneapolis, MN, USA and LEGEND MAX™; Biolegend, San Diego, CA, USA respectively). Because of the limited sample volume, we performed the experiments with single well for each sample. In brief, pre-coated plates were serially incubated with CSF or serum samples, detection antibodies and appropriate chromogens in the dark. Plates were sealed and placed in orbital shaker (250 rpm) at room temperature during incubation steps and extensively washed between incubation steps. Optical density of each well was determined using a microplate reader (Multiskan MK3, Thermo electron corporation, USA) set at 450 nm. Serial dilutions of recombined BAFF and APRIL proteins were used as standards, and standard curves were generated to calculate protein concentrations.

2.3. Statistical analysis

Statistical analysis were performed using SPSS 17.0 and GraphPad Prism 5 software. The data were expressed as mean ± SD (normally distributed data) or median with range (non-normally distributed data). BAFF and APRIL concentrations were log-transformed due to skewed distribution. Comparisons between two groups were made by independent or paired *t*-test. One-way ANOVA with Dunnett's T3 post hoc test was applied to assess difference of BAFF and APRIL concentrations in CSF samples of control group and patients in different disease stages. Nonparametric testing was performed by the Mann-Whitney *U* test. Frequency comparisons were performed using chi-square test or Fisher's exact test. Correlation analysis was determined by Spearman's rank correlation coefficient. Difference at the $p < 0.05$ level was considered statistically significant.

3. Results

3.1. Clinical characteristics and demographics

The clinical features of patients with anti-NMDAR encephalitis and controls are listed in Table 1. Age and gender of patients and controls had no significant difference. Twenty (50%) patients were female, and 9 (22.5%) patients were accompanied by ovary teratomas. All patients presented with typical symptoms of anti-NMDAR encephalitis, of which memory deficit, seizures, abnormal behavior and psychiatric symptoms were most common symptoms. Only 13 (32.5%) patients had abnormal brain MRI. On CSF examinations, CSF WBC count and protein levels were increased in patients compared to controls ($p < 0.001$ and $p < 0.05$, Table 1). All patients received at least one immunotherapy. After treatment, 27 (67.5%) patients achieved favorable outcome (mRS < 2).

3.2. BAFF and APRIL were increased in CSF of patients with anti-NMDAR encephalitis

The levels of both BAFF and APRIL in the CSF of 40 patients with anti-NMDAR encephalitis and 20 control individuals were measured. The BAFF concentrations in CSF were significantly increased in patients with anti-NMDAR encephalitis compared to control individuals (99.3 ± 2.5 vs. 48.6 ± 1.3 pg/mL, $p < 0.001$; Fig. 1A). We also found that mean APRIL level in CSF in patients' group was also significantly higher than that in control group (3.7 ± 1.7 vs. 2.5 ± 1.3 ng/mL, $p < 0.001$; Fig. 1B). BAFF and APRIL concentrations in the serum of patients and controls had no significant difference (BAFF: 808.4 ± 1.9 vs. 869.7 ± 1.3 pg/mL; APRIL: 32.5 ± 1.4 vs. 27.3 ± 1.5 ng/mL, all $p > 0.05$; Fig. 1C and D).

3.3. Correlation analysis of BAFF and APRIL in CSF and serum

The levels of BAFF and APRIL were positively correlated in CSF of patients with anti-NMDAR encephalitis ($r = 0.409$, $p < 0.01$; Fig. 2A). No correlation was found between serum BAFF and APRIL ($r = -0.108$, $p > 0.05$; Fig. 2B). Furthermore, we analyzed paired CSF and serum to investigate whether systemic circulating BAFF and APRIL levels had influence on CSF of these two cytokines. The results showed serum and CSF