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Short Communication

Cerebrospinal fluid pentraxin 3 and CD40 ligand in anti-*N*-menthyl-D-aspartate receptor encephalitis

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ABSTRACT

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune disorder of the central nervous system whose pathogenesis involves interleukin (IL)-6 and IL-17A. We examined the correlations between CSF concentrations of the acute-phase protein pentraxin 3 (PTX3), the chronic inflammatory mediator CD40L, IL-6, and IL-17A in anti-NMDAR encephalitis, and the impact on clinical outcome. PTX3, CD40L, IL-6, and IL-17A were significantly higher in the CSF of patients with anti-NMDAR encephalitis than in controls. Within the former, PTX3 levels correlated positively with IL-6 and the mRS, and CD40L levels with IL-17A and the mRS. Higher PTX3 and CD40L levels may reflect the underlying neuroinflammation.

1. Introduction

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune disorder of the central nervous system (CNS) that predominantly affects young females (Titulaer et al., 2013). Anti-NMDAR is one of the most commonly detected antibodies in autoimmune encephalitis, comprising approximately 80% of the total antibodies in this disease (Lancaster et al., 2011). The typical clinical manifestation of anti-NMDAR encephalitis is the progressive multistage development of psychiatric and neurologic symptoms, such as seizures, abnormal movements, autonomic disturbances, and disorders of memory, behavior, and cognition (Vincent and Bien, 2008; Dalmau et al., 2007). However, although the transformation of B and T leukocytes may be involved in this disease, its immunopathogenesis has yet to be fully elucidated (Tüzün et al., 2009).

Pentraxin 3 (PTX3), a member of the long pentraxins, plays an important role in innate immunity, vascular inflammation, and extracellular matrix functionality in the peripheral circulation (Manfredi et al., 2008; Mantovani et al., 2006; Salustri et al., 2004). It is produced by vascular cells in response to inflammatory signals, including tumor necrosis factor-α (TNF-α), interferon-1β, lipopolysaccharide, and other agonists of different members of the Toll-like receptor family (Mantovani et al., 2006; Alles et al., 1994; Basile et al., 1997), and is therefore an indicator of the inflammatory response. PTX3 is induced following pathogen recognition and therefore acts at an early stage in the immune response (before the production of antibodies); it promotes complement activation and, as a result of opsonization, phagocytosis (Maina et al., 2009; Nauta et al., 2003; Cotena et al., 2007). High PTX3 levels are associated with acute myocardial infarction, ischemic stroke, and Parkinson's disease (Muller et al., 2001; Sciacca et al., 2010; Ryu et al., 2012) but whether increased levels occur in anti-NMDAR encephalitis patients has not been determined.

CD40 ligand (CD40L, formally known as CD154), a 33-kDa type II membrane glycoprotein from the TNF-α family, is mainly expressed on activated T cells and platelets. Like other members of the TNF family, CD40L is detected in soluble form (sCD40L), the major source of which is activated platelets (Andre et al., 2002; Danese and Fiocchi, 2005). Elevated levels of sCD40L are found in a variety of diseases in which the ligand has been implicated in the initiation or potentiation of inflammation, such as cardiovascular disease, atherosclerosis, inflammatory bowel syndrome, fibrosis, and type 1 diabetes (Tsakiris et al., 2000; Heeschen et al., 2003; Devaraj et al., 2006). However, whether elevated CD40L levels are also a feature of anti-NMDAR encephalitis had not been determined.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; IL, interleukin; NMDAR, *N*-methyl-D-aspartate receptor; PTX, pentraxin; SD, standard deviation; TNF, tumor necrosis factor; Th, T helper

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Table 1
Clinical data of patients with anti-NMDA receptor(NMDAR)encephalitis and non-inflammatory neurological disorder controls.

	NMDAR	Control
No. of patients	24	31
Gender (male/female)	14/10	15/16
Age (years)	36.42 ± 17.42	37.42 ± 15.27
Anti-NMDAR antibody	24	0
mRS score	2.87 ± 0.90	–

Because T helper (Th) cells support B cells (Muranski and Restifo, 2013), in this study we measured the levels of Th cell cytokines that support Th cell function, including interleukin (IL)-6 and IL-17A (Damsker et al., 2010). Also, as susceptibility to neuroinflammation may contribute to the severity of anti-NMDAR encephalitis, we examined whether the levels of CSF inflammatory cytokines as well as PTX3 and CD40L were increased in anti-NMDAR encephalitis patients. We then investigated the association between pro-inflammatory factors and this neuroinflammatory disorder.

2. Materials and method

The study population consisted of 24 patients and 31 controls prospectively recruited from The Department of Neurology, Nanfang Hospital of Southern Medical University. The inclusion criteria for the patient group were based on the revised criteria for the diagnosis of anti-NMDAR encephalitis published by Graus et al. in 2016 (Graus et al., 2016). In all 24 patients, CSF samples were positive for anti-NMDAR antibodies at the time of diagnosis. The control group consisted of 31 age- and sex-matched patients with other non-inflammatory neurological disorders, including cerebrovascular diseases and movement disorders, but whose CSF inflammatory cytokine levels were not indicative of a diagnosis of anti-NMDAR encephalitis.

The CSF samples were stored at − 80 °C until the assays were performed. CSF levels of the inflammatory cytokines PTX3 and CD40L were measured using enzyme-linked immunosorbent assays (R&D Systems, McKinley Place NE, Minneapolis, MN, USA). The main clinical outcomes were evaluated using the modified Rankin scale (mRS) at 3 months after ictus. The study was conducted with the approval of the Ethics Committee of The Nanfang Hospital, Southern Medical University.

All data were statistically analyzed using SPSS version 20.0 (IBM Corp, Armonk, NY, USA). The data are expressed as the mean ± standard deviation (SD). Independent-samples *t*-tests were used to compare the CSF levels of inflammatory cytokines, PTX3, and CD40L in the anti-NMDAR encephalitis group and the controls. Correlations between parameters were calculated using the Pearson test. A P value < 0.05 was considered to indicate statistical significance.

3. Results

The baseline characteristics of the patient and control groups are shown in Table 1. The mean age of the patients with anti-NMDAR antibodies was 36.42 ± 17.42 years, and that of the control group 37.42 ± 15.27 years. The difference was not significant, nor was the sex distribution between the two groups. The mean mRS of the patients was 2.87 ± 0.90.

The mean PTX3 concentration (ng/mL) of the 24 patients with anti-NMDA receptor encephalitis and the 31 controls was 6.93 ± 4.96 and 4.94 ± 1.33, respectively. CSF- PTX3 levels were higher ($P = 0.037$) in the patients (Fig. 1B). The mean CD40L, IL-6, and IL-17A concentrations (ng/mL) in the CSF of patients with anti-NMDAR encephalitis were 1063.21 ± 682.25, 5.95 ± 4.61, and 9.70 ± 6.75, respectively, and were significantly higher than in the controls (560.71 ± 200.32, 3.43 ± 1.31, and 3.58 ± 1.54, respectively;

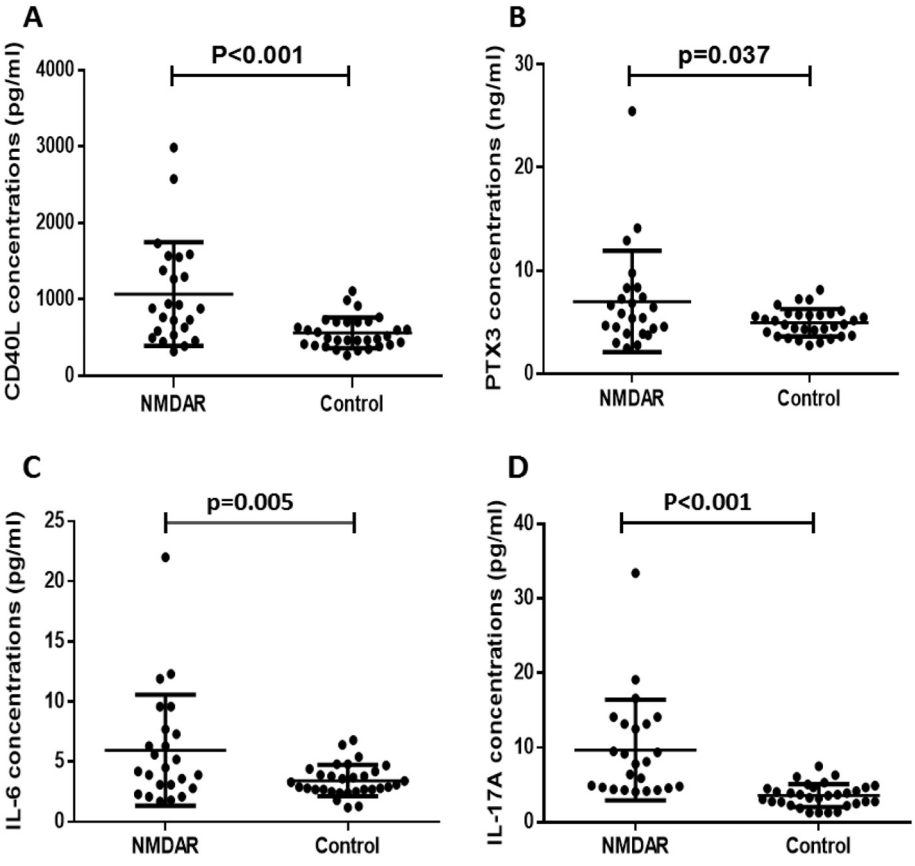

Fig. 1. Cerebrospinal fluid levels of CD40L, pentraxin 3 (PTX3), interleukin (IL)-6 and IL-17A.

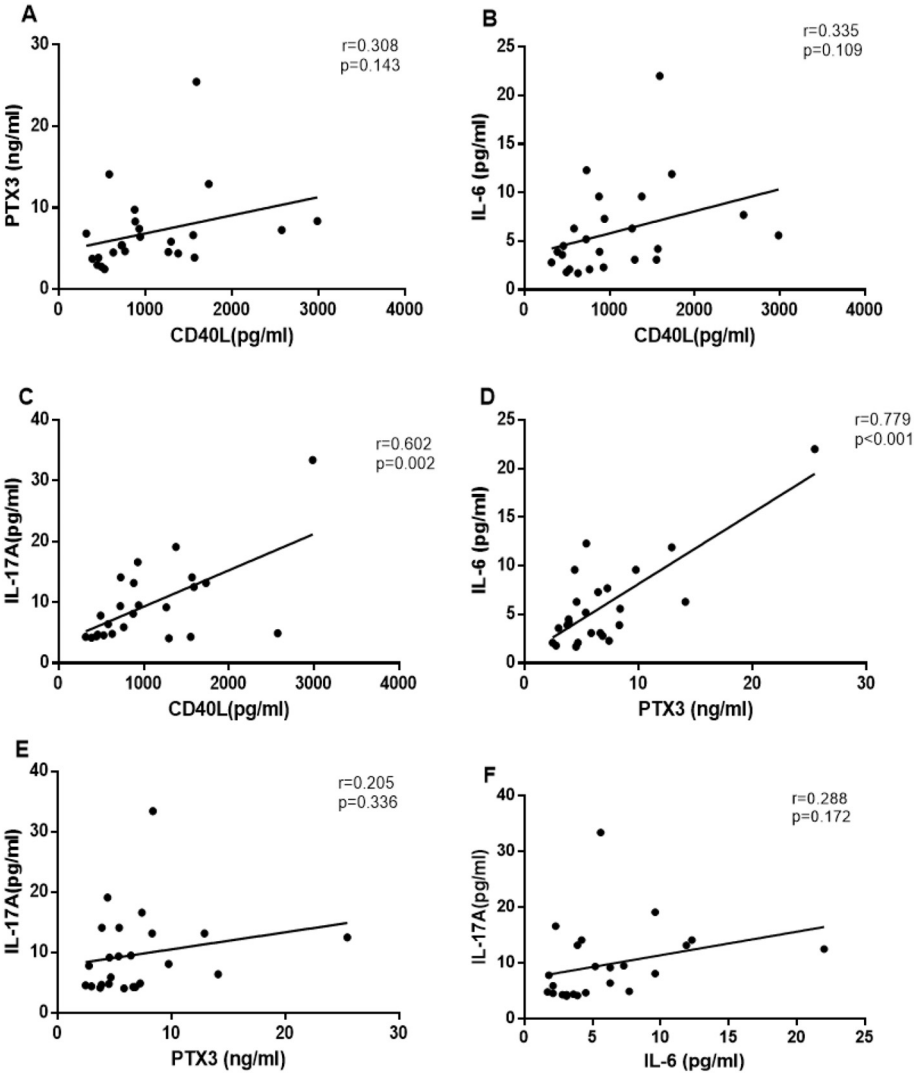

Fig. 2. CSF levels of PTX3 and CD40L correlate positively with those of IL-6 and IL-17 in patients with anti-NMDAR encephalitis.

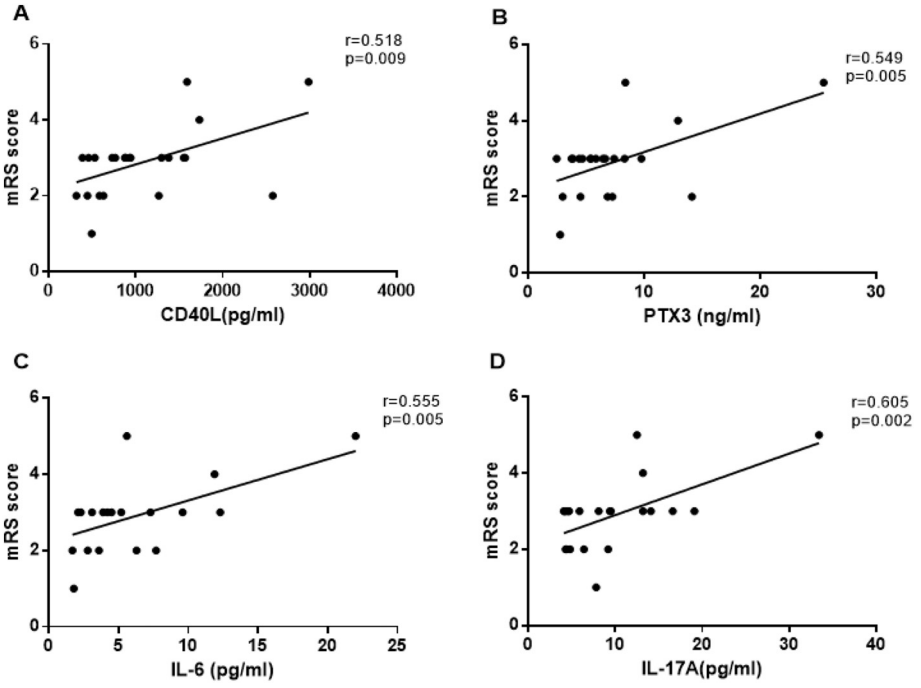

Fig. 3. CSF levels of IL-6, IL-17A, PTX3, and CD40L in CSF correlate positively with the mRS scores of patients with anti-NMDAR encephalitis.

$P < 0.05$) (Fig. 1A, C, D).

An analysis of the relationships between CD40L, PTX3, IL-6, and IL-17A levels revealed positive correlations between CD40L and IL-17A as well as between PTX3 and IL-6 in patients with anti-NMDAR encephalitis, with correlation coefficients of 0.602 and 0.779, respectively ($P < 0.05$) (Fig. 2C, D), but not in the control group (data not shown). There were no correlations between any of the other measured pro-inflammatory factors/cytokines (Fig. 2A, B, E, F). However, the correlations between CSF levels of IL-6, IL-17A, PTX3, CD40L, and mRS in the NMDAR group were significant ($P < 0.05$), with correlation coefficients of 0.518, 0.549, 0.555, and 0.605, respectively (Fig. 3A–D). The CSF levels of any of the inflammatory cytokines did not correlate with the age or sex of the anti-NMDAR encephalitis patients (data not shown).

4. Discussion

Inflammatory diseases of the CNS present a diagnostic challenge to clinicians. Anti-NMDAR encephalitis, an anti-neuronal antibody-mediated inflammatory disease of the brain, is an important diagnostic consideration in patients presenting with characteristic progressive neuropsychiatric symptoms and non-specific evidence of CNS inflammation (Rosenfeld et al., 2010). Elevated serum inflammatory markers and CSF pleocytosis are commonly found in patients with this disease and may be useful diagnostic indicators. Early recognition of anti-NMDAR encephalitis is crucial, since it is treatable and early treatment is tightly linked to a better prognosis (Wandinger et al., 2011).

Our cross-sectional study showed significantly higher CSF levels of PTX3, CD40L, IL-6, and IL-17A in patients with anti-NMDAR encephalitis than in controls. PTX3, an acute-phase protein, plays an important role in inflammation. Under inflammatory conditions, it is secreted by reactive glial cells and can be detected in serum. Higher serum PTX3 levels are a predictor of cognitive impairment (Yano et al., 2010) and are involved in autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, and autoimmune disease (Huang et al., 2016).

CD40L, a member of the TNF-α family expressed predominantly by activated CD4 + T cells and platelets (Li, 2008), participates in a variety of pro-inflammatory and neurologic diseases in the brain. Aberrant expression of CD40L has been detected in HIV-1-associated dementia (D'Aversa et al., 2002), multiple sclerosis (Gerritse et al., 1996), and Alzheimer's disease (Ait-Ghezala et al., 2005).

The pro-inflammatory cytokine IL-17A is produced from type 17 helper T cells. Elevated IL-17A levels have been measured in the CSF of patients with an opticospinal form of multiple sclerosis (Ishizu et al., 2005) and neuromyelitis optica (NMO) spectrum disease (Matsushita et al., 2013). IL-6 is another pro-inflammatory cytokine elevated in the CNS of patients with autoimmune diseases, including NMO (Uzawa et al., 2010). Our results showed that the CSF levels of PTX3, CD40L, IL-6, and IL-17A were significantly higher in anti-NMDAR encephalitis patients than in controls, suggesting their active participation in anti-NMDAR encephalitis.

Moreover, positive correlations were also detected between PTX3 and IL-6 and between CD40L and IL-17A in the CSF of anti-NMDAR encephalitis patients. During inflammation, the response to inflammatory stimuli is characterized by the generation of a chemokine gradient by cells secreting IL-1, IL-6, granulocyte-macrophage colony-stimulating factor, and TNF-α. Shiraki et al. showed that the stimulation of macrophages with pathological concentrations of PTX3 inhibits pro-inflammatory signaling and cytokine production (Shiraki et al., 2016), supporting the notion that PTX3 is an anti-inflammatory protein. Its production following inflammatory insult may stimulate the anti-inflammatory response (Slusher et al., 2016).

IL-17A is a pro-inflammatory cytokine produced from type 17 helper T cells, while CD40L and its receptor, CD40, play a crucial role in immunity, including the expression of genes related to cellular stress, the activation and differentiation of immune cells, T-cell activation during graft rejection, and autoimmunity (Chatzigeorgiou et al., 2009). These features of CD40L and IL-17A may explain their positive correlation in anti-NMDAR encephalitis.

The mRS is often used to evaluate neurological recovery and to measure outcomes of disease. The positive correlations between CSF PTX3, CD40L levels, and clinical outcomes, according to the mRS, suggests that measuring CD40L and PTX3 concentrations can contribute to assessing the prognosis of patients with anti-NMDAR encephalitis. However, further studies are needed to fully elucidate the nature of this disease.

5. Conclusion

This study showed markedly higher levels of PTX3, CD40L, IL-6, and IL-17A in the CSF of patients with anti-NMDAR antibodies. The levels of PTX3, CD40L, and the cytokines IL-6 and IL-17A in the CSF correlated positively with the clinical outcome of patients with anti-NMDAR encephalitis. These results suggest that the higher levels of PTX3 and CD40L levels in the CSF of patients with this disease reflect the underlying processes of this neuroinflammatory disorder and a relationship to its clinical outcome.

Consent for publication

Not applicable.

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Competing interests

The authors have no competing interests to declare.

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