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Short communication

Serum concentration of CD40L is elevated in inflammatory demyelinating diseases

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ABSTRACT

It is believed that auto-inflammatory activity, including cellular and humoral immunity responses, especially T cell–B cell collaboration, is one of the most important components of the pathogenesis of inflammatory demyelinating disease. CD40L is critical for T cell–B cell collaboration. Actually, serum CD40L levels have been shown to increase in MS. In the present study, serum CD40L levels were measured by an enzyme-linked immunosorbent assay (ELISA) in NMO ($n = 27$) and MS ($n = 19$) patients and controls ($n = 14$). We revealed elevation of CD40L in NMO patients, and discovered a correlation between CD40L and humoral immunity in inflammatory demyelinating disease.

1. Introduction

Neuromyelitis optica (NMO) and multiple sclerosis (MS) are two typical autoimmune inflammatory demyelinating diseases of the central nervous system (CNS), and they share many similarities. For several years, it has been a matter of debate whether NMO is a distinct disease entity or a variant of MS. Until 2004, serum anti-aquaporin 4 antibody (AQP4-Ab) was thought to be a specific biomarker of NMO, distinguishing it from MS (Lennon et al., 2004). However, the pathogenesis of MS and NMO remains unclear, and it is believed that auto-inflammatory activity, including cellular and humoral immunity responses, especially T cell–B cell collaboration, is one of the most important components of the pathogenesis of these diseases.

Clinical and laboratory-based studies support a prominent role for T cells in MS pathogenesis. A cluster of differentiation 4-positive ($CD4^+$)–T-helper (Th) lymphocyte subset called Th17 has emerged as a key player in the pathogenesis of MS (Rostami et al., 2013). Simultaneously, B cells may also play a role in MS pathogenesis. Lymphoid follicle-like structures have been found in brain meninges of patients with MS (Prineas, 1979; Serafini et al., 2004; Magliozzi et al., 2007). Additionally, clonally expanded B cells are present in the CNS and cerebrospinal fluid (CSF), pointing to an antigen-driven B-cell response within the CNS (Link et al., 1977). These B cells are involved in the production of IgG within the brain compartment, giving rise to oligoclonal bands (Link et al., 1977), a hallmark of this disease (Hoffmann et al., 2014).

Convergent results have indicated that B cells play a fundamental role in NMO immunopathology (Bennett et al., 2015). The discovery of AQP4-Ab (Lennon et al., 2004) was a milestone in defining this disease entity. Rituximab directly depletes B cells and reduces the frequency of disease relapse (Kim et al., 2013). Furthermore, a series of studies has provided evidence for the important role of T cell–B cell collaboration in various steps of NMO pathogenesis. In our previous studies, we observed that the Th17 proportion and interleukin (IL)-17A level were elevated in patients with NMO (Li et al., 2011); later in our studies, other relative T cell subtypes were found to be involved in the pathogenetic mechanisms of NMO (Wang et al., 2011; Xu et al., 2013). Most importantly, the increasing evidence of extensive crosstalk (in both directions) between Th17 and B cells is consistent with our findings and supports the role of T cell–B cell collaboration in NMO pathogenesis (Mitsdoerffer et al., 2010).

An important receptor–ligand pair involved in the regulation of the immune response is CD40 and its ligand, CD40L. CD40 is a tumor necrosis factor receptor superfamily member expressed by immune and non-immune cells. Its ligand, CD40L, is transiently expressed on the surface of activated $CD4^+$-T cells, but can also be upregulated on other cell types in autoimmune diseases (Katsiari et al., 2002; Grammer et al., 2003). Originally reported to be crucial for the activation and maturation of B cells in the humoral response, it is becoming increasingly

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clear that the CD40/CD40L interaction is also of key importance for Th-cell activation and Th-induced activation of effector cells. Moreover, CD40/CD40L signaling results in production of pro-inflammatory cytokines, such as IL-6, which can influence T-cell differentiation to Th17 cells (Iezzi et al., 2009). Consequently, CD40L is an attractive candidate for contributing to a variety of autoimmune processes in which T cell–B cell collaboration plays a role in their pathogenesis, for example, MS and NMO (Peters et al., 2009).

Serum CD40L levels have been shown to increase in MS and to be significantly reduced after effective treatment (Carrieri et al., 2015). However, whether levels of CD40L are elevated in patients with NMO remains unknown. Therefore, the aim of the present study was to measure the serum concentration of CD40L in patients with NMO and to determine the relationship of CD40L with demyelinating disease activity.

2. Participants and methods

2.1. Patients and controls

Twenty-seven patients diagnosed with NMO based on the 2015 diagnostic criteria (Wingerchuk et al., 2015) along with 19 patients with relapsing–remitting MS fulfilling the 2010 McDonald's diagnostic criteria (Polman et al., 2011) were enrolled from the demyelinating disease database of the Neurology Department of the Third Affiliated Hospital of Sun Yat-sen University. Patients enrolled from the database were diagnosed by two neurologists. Fourteen volunteer healthy controls (CTLs) were recruited. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. Written informed consent was obtained from each participant.

The patients with MS who were enrolled were naïve to disease-modifying treatments. Three of the patients with NMO received small doses of steroid hormone plus azathioprine treatment during remission, but they discontinued treatment and relapsed before being admitted to our hospital. All samples were obtained during the relapse phase prior to the start of treatment. A clinical relapse was defined as a sudden appearance of new symptoms, lasting for at least 24 h, with an increase of over 1.0 in the Expanded Disability Status Scale (EDSS) score. Demographic and clinical features of the patients and healthy controls are shown in Table 1. There were no significant differences in age and gender between the groups.

2.2. Preparation of blood samples

All blood samples were centrifuged for 15 min at 1000 $\times g$ within 30 min of collection to eliminate cells and other insoluble materials and then aliquoted into polypropylene tubes for storage at −80 °C.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Serum CD40L concentrations were measured using commercially available ELISA kits (R&D Systems, Abingdon, UK) according to the manufacturer's instructions. Samples in which the CD40L concentration exceeded the detection range were serially diluted in assay diluent until they reached the dynamic range of the assay. The mean minimum detectable concentration of CD40L was 4.2 pg/mL. The goodness of fit for the representative standard curve was $r^2 = 0.993$.

Table 1
Demographic and clinical features of the patients and controls.

	NMO ($n = 27$)	MS ($n = 19$)	CTLs ($n = 14$)
Gender, female/male	25/2	15/4	11/3
Age (years)	36.0 (11–59)	32.0 (20–55)	32.5 (27–46)
Onset age (years)	30.0 (8–57)	28.0 (16–54)	N/A
Disease duration (years)	2.0 (1 − 20)	1.5 (0.8–15)	N/A
EDSS	4.0 (2.0–9.0)	3.0 (2.0–7.5)	N/A
Annualized relapse rate	1.67 (0.15–6.0)	1.00 (0.2–3.75)	N/A
NMO-IgG, positive/negative	25/2	0/18	N/A

Age (years) refers to age at sampling time point.
Disease duration (years) refers to years from disease onset to sampling.
CTLs, controls; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; N/A: not available; NMO, neuromyelitis optica.

2.4. Detection of immune response indexes

The hospital laboratories of the Third Affiliated Hospital of Sun Yat-sen University determined the serum levels of the following: cellular immune response indicators, including $CD4^+$-T cells, $CD8^+$-T cells; humoral immune response indicators, including C-reactive protein (CRP), immunoglobulins (IgG, IgA, and IgM), and complements (C3, C4, and CH50); erythrocyte sedimentation rate (ESR); thyroid autoimmune antibodies and other immune antibodies.

2.5. AQP4-Ab detection

Indirect immunofluorescence test systems for human AQP4-Ab detection from EUROIMMUN (Euroimmun Medizinische Labordiagnostika, Lübeck, Germany) were used according to the manufacturer's instructions.

2.6. Statistical analysis

The data are presented as the mean ± standard deviation (serum CD40L, $CD4^+$-T cells, $CD8^+$-T cells, CRP, IgG, IgA, IgM, C3, C4, CH50, and ESR) or the median with range (age, onset age, disease duration, annualized relapse rate [ARR], and EDSS score). Differences in concentrations of serum CD40L between different subgroups were analyzed using the Mann–Whitney *U* test. Correlations between CD40L and clinical or immune response indicators were analyzed using Spearman's rank correlation. *P* values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) for windows.

3. Results

3.1. Serum CD40L concentrations in patients with MS or NMO and in CTLs

The concentration of CD40L was determined in serum from patients with NMO ($n = 27$) or with MS ($n = 19$) and in CTLs ($n = 14$) using ELISAs. The mean CD40L concentrations (pg/mL) for patients with NMO or MS and CTLs were 3087.30 ± 790.27, 2878.10 ± 606.56, and 2200.60 ± 938.53, respectively. The CD40L serum concentration was significantly higher in patients with inflammatory demyelinating diseases than in CTLs (NMO, $P = 0.004$; MS, $P = 0.050$). A comparison of CD40L serum concentrations within the two subgroups of inflammatory demyelinating diseases showed that the concentration of CD40L was slightly higher in the NMO group than in the MS group, but this trend was not statistically significant ($P = 0.255$; Fig. 1).

3.2. Serum CD40L concentrations in different subgroups

Clinical data, including age, sex, ARR, and EDSS scores, from all patients with NMO or MS are presented in Table 1. The serum CD40L concentration appeared to be higher in females than in males in all subgroups, but there was no significant difference (NMO, $P = 0.459$; MS, $P = 0.764$; CTLs, $P = 0.275$). Additionally, the other clinical indicators examined (age, ARR, and EDSS score) were not correlated with the concentration of serum CD40L.

3.3. Relation between serum CD40L concentration and the cellular immune response

The cellular immune response was assessed in NMO ($n = 12$) and MS patients ($n = 9$). No significant correlation was detected between