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Decreased serum IL-27 and IL-35 levels are associated with disease severity in neuromyelitis optica spectrum disorders

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ABSTRACT

The interleukin 12 (IL-12) family plays important roles in autoimmune diseases. To explore the roles of the IL-12 family members IL-27 and IL-35 in the pathogenesis of neuromyelitis optica spectrum disorders (NMOSD), we determined their serum and cerebral spinal fluid levels and assessed potential correlations with clinical characteristics. Serum IL-27 levels were negatively correlated with disease severity and spinal cord lesion length, while serum IL-35 levels were negatively correlated with disease severity and annual relapse rate. Thus, IL-27 and IL-35 may be important biomarkers of NMOSD severity and these molecules might represent potential therapeutic cytokines for treating NMOSD.

1. Introduction

Neuromyelitis optica spectrum disorders (NMOSD) have been defined as rare, disabling autoimmune spectrum diseases of the central nervous system, which are distinct from multiple sclerosis (MS). NMOSD comprise neuromyelitis optica (NMO), longitudinally extensive myelitis (LETM), optic neuritis (ON), and other typical demyelinating brain syndromes involving the area postrema, periependymal brainstem, diencephalon, and cerebrum (Wingerchuk et al., 2015). Great progress in our understanding of the pathogenesis of NMOSD has been made since the discovery that aberrant anti-aquaporin 4 antibodies (AQP4 Ab) in the serum of patients with NMOSD underlie many disease phenotypes (Jarius and Wildemann, 2010; Lennon et al., 2004).

In addition to AQP4 Ab, recent studies have demonstrated that various cytokines and chemokines may be involved in immune responses and pathogenesis of the peripheral/central nervous system in patients with NMO such as IL-6, IL-17, IL-10, IL-12, IFN-γ, BAFF, CXCL13 and TNF-α(Matsushita et al., 2013; Uzawa et al., 2010, 2014; Zhong et al., 2011). However, the role of newly recognized members of the interleukin12 (IL-12) family, such as IL-27 and IL-35, remains unclear. It is known that members of the IL-12 family, including IL-12, IL-23, IL-27 and IL-35, play important roles in regulating immune responses and the pathology of inflammatory and autoimmune diseases (Sun et al., 2015; Vignali and Kuchroo, 2012).

Recent studies have demonstrated that IL-12 and IL-23 are pro-inflammatory cytokines that are mainly secreted by dendritic cells (DCs) and macrophages. In turn, these molecules can induce the production of a number of cytokines, regulate a variety of effector cells, and contribute to the pathogenesis of CNS autoimmune diseases(Becher et al., 2003; Comabella et al., 1998; Cua et al., 2003; Vignali and Kuchroo, 2012). IL-27 is a newly recognized member of the IL-12 family composed of Epstein–Barr virus-induced gene protein 3 (EBI3) and p28 subunits, which is often produced by activated antigen presenting cells. Intriguingly, IL-27 can play both pro-inflammatory and anti-inflammatory roles(Batten et al., 2006; Cox et al., 2011; Fitzgerald et al., 2007). IL-35 is similar to IL-27 in that it is a heterodimeric cytokine encompassing the p35 subunit of IL-12 and EBI3. IL-35 is secreted by activated inflammatory cells, such as anti-CD3/CD28-induced Treg cells, activated dendritic cells, and macrophages. IL-35 has been shown to perform an immunomodulatory role in a wide variety of disease conditions(Collison et al., 2010, 2007; Wang et al., 2014).

However, possible roles of IL-27 and IL-35 in NMOSD are unknown. Therefore, in the present study, we determined the levels of IL-27 and IL-35 in the serum and cerebrospinal fluid (CSF) of patients with NMOSD and explored if there were relationships between these two newly recognized IL-12 members and clinical parameters.

2. Methods

2.1. Subjects

Forty-five patients with NMOSD were recruited from the Neurology Department of Tianjin Medical University General Hospital from January 2012 to July 2015. The patients were diagnosed according to

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the criteria defined by Wingerchuk et al.(Wingerchuk et al., 2015). All patients with NMOSD were treated with intravenous methylprednisolone at a dose of 0.5 g/day for 3–5 consecutive days during acute exacerbations, and then a subset were treated with low-dose prednisolone and/or azathioprine at a dose of 0.1 g/day during the initial two years of remission phases. As a serum control group, we enrolled forty healthy age- and sex-matched individuals from the Health Care Center of our hospital. In addition, we enrolled 19 patients with other non-inflammatory neurological disorders (ONNDs) as CSF controls, comprising 6 patients with subacute combined degeneration of the spinal cord, 11 patients with motor neuron disease, and 2 patients with Wernicke–Korsakoff syndrome.

All the research procedures were approved by the ethics committee of Tianjin Medical University General Hospital, and all the participants provided written informed consent prior to participation.

2.2. Clinical assessment

The data for demographic features, clinical symptoms, Kurtzke Expanded Disability Status Scale (EDSS) score at sampling, annual relapse rate during the disease course (number of relapses per year), and CSF findings including cells, protein and oligoclonal bands, were acquired from our internal database system. The EDSS was evaluated independently by two neurologists who were certified for competency in EDSS scoring by Neurostatus (Neurostatus Systems AG, Basel, Switzerland). Conventional and/or enhanced MRI of brain and spinal cord were all performed within 7 days of attack to identify new lesions.

A total of 45 serum samples and 21 CSF samples matched with serum samples were obtained during acute exacerbations. Twelve serum and 9 CSF samples were collected at the patient's first NMOSD attack. All the serum samples were collected before high-dose intravenous methylprednisolone for acute exacerbations; 26 of these samples were collected from patients who had not received low-dose oral prednisolone and/or azathioprine therapy within at least three months before starting with sampling. Only 10 CSF samples were collected before the administration of any immunosuppressive therapy. All samples were stored at −80 °C until analysis.

2.3. Detection of anti-AQP4 antibodies

Serum AQP4 antibody was detected by both CBA and fluorescence immunoprecipitation assay (FIPA)(Wang et al., 2015; Yang et al., 2014). The EGFP tagged human AQP4 plasmid and the EGFP plasmid were donated by Professor Angela Vincent and Professor David Beeson (Nuffield Department of Clinical Neurosciences, University of Oxford).

2.4. Measurement of IL-27and IL-35 levels

Serum and CSF IL-27 levels were measured using a human IL-27 enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. We also detected serum and CSF IL-35 levels with a human IL-35 enzyme-linked immunosorbent assay (ELISA) kit (Biolegend, San Diego, CA,USA) according to the manufacturer's instructions. The absorbance values of the corresponding substrate were read at 450 nm. IL-27 and IL-35 levels were calculated by referencing to a standard curve, and the lowest detectable levels were 9.5 pg/mL and 0.13 ng/mL, respectively. If the serum or CSF levels were below the lowest detectable levels, we recorded the concentration of serum or CSF IL-27 or IL-35 as zero.

2.5. Statistical analyses

Unpaired t-tests or Mann–Whitney U tests were used for comparisons of continuous variables. Spearman's rank correlation analysis was used for analysis of the associations between IL-27 or IL-35 and clinical parameters. All statistical analyses were performed using GraphPad PRISM 5.0 software (GraphPad Software Inc., San Diego, CA, USA). $p < 0.05$ was considered to be statistically significant in two-tailed tests.

3. Results

3.1. Demographic and clinical features of subjects with NMOSD

The presenting demographic and clinical features of the 45 patients with NMOSD included in the study are shown in Table 1. All subjects belonged to the Chinese Han ethnic group.

3.2. Serum and CSF IL-27 and IL-35 levels in NMOSD

We determined IL-27 and IL-35 levels in patients with NMOSD and healthy controls to explore the role of IL-27- and IL-35-mediated inflammation in the pathogenesis of NMOSD. Our results indicated that serum IL-27 levels were significantly decreased in patients with NMOSD compared to controls ($p < 0.001$, Fig. 1). Given that subset of the enrolled patients had received low-dose oral immunosuppressive treatments before sampling, we compared IL-27 levels in the medicated versus unmedicated groups prior to sampling. We found that IL-27 levels in the medicated group were higher than in the unmedicated group, but the difference did not meet the level of statistical significance ($p = 0.168$). We further analyzed serum IL-27 levels in anti-AQP4 antibody-positive patients and anti-AQP4 antibody-negative patients, we found that serum IL-27 levels were similar between these two groups ($p = 0.338$). IL-27 levels in both the anti-AQP4 antibody-positive and anti-AQP4 antibody-negative groups were lower than in control subjects ($p = 0.004$, $p = 0.005$, respectively, Fig. 1). Similarly, we found that serum IL-35 levels in patients with NMOSD were significantly lower than in controls ($p = 0.009$, Fig. 2), and there was no statistical difference of IL-35 levels between the medicated and unmedicated groups before sampling ($p = 0.079$). Serum IL-35 levels in anti-AQP4 antibody-positive patients were significantly decreased compared to control subjects ($p = 0.017$, Fig. 2). Although serum IL-35 levels in anti-AQP4 antibody-negative patients were also lower than in control subjects, the difference was not statistically significant ($p = 0.096$). There was likewise not a significant difference in serum IL-35 levels between patients with anti-AQP4 antibody-positive and anti-AQP4 antibody-negative findings ($p = 0.942$). CSF IL-27 and IL-35 levels were both below the minimum detectable level in patients with NMOSD and in control subjects.

Table 1
Characteristics of subjects with NMOSD.

Clinical characteristics	NMOSD (n = 45)
Age at sampling (years)	46.71 ± 13.24 (20–74)
Sex (F:M)	37:8
Disease duration at the last follow-up (years)	8.80 ± 6.83 (2.00–36.00)
Disease duration at sampling (years)	4.38 ± 6.08 (0–24.00)
Annual relapse rate during the disease course (number of relapses per year)	0.58 ± 0.33
EDSS at sampling	4.90 ± 2.01
Attack sites at sampling	
No. of patients with optic neuritis	4/45 (8.89%)
No. of patients with acute myelitis	45/45 (100%)
No. of patients with intracranial lesions	11/45 (24.44%)
MRI total length of newly identified spinal cord lesion length at sampling (vertebral segments)	6.60 ± 4.09 (2–18)
No. of patients with optic neuritis	35/45 (77.78%)
No. of patients with MRI cerebral lesions	31/43 (72.09%)
No. of patients with serum AQP4 antibody-positive at sampling (%)	36/45 (80.00%)
CSF (no.)	21/45
No. of cell (per μL)	30.10 ± 73.44 (0–276)
Protein (mg/dL)	45.90 ± 22.06
No. of patients with oligoclonal band-positive at sampling	1/21