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Increased serum concentrations of transforming growth factor-β1 (TGF-β1) in patients with Guillain-Barré syndrome

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ABSTRACT

Background: Guillain-Barré syndrome (GBS) is an acquired demyelinating peripheral neuropathy. It has shown that macrophage activation contribute to the pathogenesis of GBS. Therefore macrophage-mediated factors could be the potential markers for disease diagnosis and status of GBS.
Methods: We measured serum concentrations of 4 macrophage-mediated factors, including interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1), vascular cell adhesion protein 1 (VCAM-1) and vascular endothelial growth factor (VEGF), in 23 chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), 28 GBS, 11 Miller-Fisher syndrome (MFS), 40 multiple sclerosis (MS), and 12 Alzheimer's disease (AD) patients, as well as 15 healthy controls.
Results: Serum TGF-β1 concentration of GBS patients (35.94 ± 2.55 ng/ml) was significantly higher compared with CIDP (25.46 ± 1.40 ng/ml, $P < 0.001$), MFS (25.32 ± 2.31 ng/ml, $P = 0.010$), MS (21.35 ± 0.90 ng/ml, $P < 0.001$) and AD patients (22.92 ± 1.82 ng/ml, $P < 0.001$), as well as healthy controls (23.12 ± 1.67 ng/ml, $P < 0.001$). A positive correlation between serum TGF-β1 concentrations and Hughes' functional grading scales was observed in GBS patients. Serum concentrations of IL-6, VCAM-1 and VEGF were similar between the studied groups.
Conclusion: The high serum concentrations of TGF-β1 and the correlation between serum TGF-β1 concentration and disease severity highlight the potential of TGF-β1 as a biomarker of GBS.

1. Introduction

Guillain-Barré syndrome (GBS) is an acquired acute inflammatory peripheral neuropathies [1]. According to different pathophysiological and clinical features, GBS can be classified into several subtypes such as acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN), and Miller-Fisher syndrome (MFS) [1]. GBS shares many symptoms and signs with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) particularly in the acute phase of disease. The time to nadir and the subsequent course of the disease are important clinical differences between these 2 diseases. GBS is a monophasic disease in which the time to reach nadir by definition is within 4 weeks [1], whereas the initial progressive phase lasts >-2 months will be considered as CIDP [2].

The diagnosis of GBS is rather based on a combination of clinical, electrophysiological features as well as analysis of the cerebrospinal fluid (CSF) at present [1]. However, the electrophysiological and CSF examinations fail to show the abnormalities at the early stage of GBS [1]. A biomarker that can be evaluated as an indicator of a pathological process or pharmacological response to a therapeutic intervention could assist in the clinical diagnosis, monitoring disease progression, and testing the efficacy of immunotherapy in GBS.

The pathogenesis of GBS is thought to be immune-mediated [3]. This immune response, possibly triggered by antecedent infection [1], may generate antibodies that cross-react with gangliosides at myelin, resulting in slowness or blockade of nerve conduction as well as damage of axons by inflammatory infiltrates [4]. Given that inflammation in GBS could be driven by peripheral lymphocytes, discovery of GBS-specific inflammatory biomarkers in peripheral blood has been repeatedly attempted. So far, a list of GBS-associated biomarkers including haptoglobin [5,6], prealbumin [7], interferon-γ [8], tumor necrosis factor-α [8], interleukin (IL)-17 [9], IL-18 [10], IL-22 [9], IL-37 [8], soluble C5b-9

Abbreviations: AD, Alzheimer's disease; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor and sensory axonal neuropathy; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CSF, cerebrospinal fluid; GBS, Guillain-Barré syndrome; IL-6, interleukin-6; INCAT disability scale, inflammatory neuropathy cause and treatment disability scale; MFS, Miller-Fisher syndrome; MS, multiple sclerosis; TGF-β1, transforming growth factor-β1; VCAM-1, vascular cell adhesion protein 1; VEGF, vascular endothelial growth factor.

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complex complement [11], matrix metalloproteinase-9 [12] and neurofilaments [13] has been disclosed in blood or CSF samples. All of these results support the critical role of peripheral inflammation in the pathogenesis GBS.

Pathologically, the inflammation of GBS may not be limited to peripheral nerves. Inflammatory infiltrates are also found in spinal cord of GBS patients [14]. The major part of inflammatory cells in GBS, activated macrophage [15], can secrete cytokines, such as IL-6 [16], transforming growth factor-β1 (TGF-β1) [16], vascular cell adhesion protein 1 (VCAM-1) [17], and vascular endothelial growth factor (VEGF) [16], which are usually associated with pro-inflammatory or anti-inflammatory responses.

2. Subjects and methods

2.1. Ethics statement

This study was performed under a protocol approved by the institutional review boards of Chang Gung Memorial Hospital (ethical license No: 103-4555C) and all examinations were performed after obtaining written informed consents.

2.2. Sample collection

Serum samples were collected from 23 CIDP (10 females, 13 males), 28 GBS (15 females, 13 males, 11 MFS (5 females, 6 males), 40 MS (28 females, 12 males), and 12 AD (7 females, 5 males) patients, as well as 15 healthy controls (7 females, 8 males). The diagnosis of GBS [18], CIDP [19], MFS [20], MS [21] and AD [22] were made according to respective diagnostic criteria. The neurological disability of the patients at the time of nadir was assessed using the Hughes' functional grading scale (for patients with GBS and MFS) [23] or Inflammatory Neuropathy Cause and Treatment (INCAT) disability scale (for patients with CIDP) [24] on the basis of the results of neurological examination and ambulatory ability assessment. All subjects were confirmed to have no systemic infection, chronic renal failure, cardiac or liver dysfunction, malignancies, or autoimmune diseases other than GBS, CIDP, MFS, MS and AD.

In the patients with GBS, MFS and MS, serum and CSF samples were collected within 2 weeks after the onset or during acute relapses of diseases. All samples were obtained before treatment with plasmapheresis, or intravenous immunoglobulins. Sample analyses were blindly performed with respect to patients' diagnosis and the results of other tests from the same patient. Serum samples were kept sitting at 4 °C for 4 h and then centrifuged, aliquoted, frozen at −80 °C, and stored until analysis. The albumin and immunoglobulin (IgG) concentrations in serum and CSF were measured by the Department of Clinical Pathology, Chang Gung Memorial Hospital.

2.3. Anti-ganglioside and anti-neuronal antigen antibodies assay

Anti-ganglioside (anti-GD1a, anti-GD1b, anti-GM1, anti-GM2, anti-GM3, anti-GQ1b and anti-GT1b) and anti-neuronal antigen (anti-AMPHIPHYSIN, anti-CV2, anti-Hu, anti-Ma2, anti-RECOVERIN, anti-Ri, anti-SOX1, anti-TITIN and anti-Yo) IgG autoantibodies were measured by using immunoblot strip kit (Euroimmun AG) according to the manufacturer's instruction.

2.4. Enzyme-linked immunosorbent assays for quantification of targeted inflammatory markers

Serum concentrations of IL-6, TGF-β1, VEGF and VCAM-1 were assessed using enzyme-linked immunosorbent assay (ELISA) (R & D Systems). Each assay was performed in duplicate according to the manufacturer's instruction.

2.5. Statistical analysis

The Statistical Program for Social Sciences (SPSS) was used to analyze all the statistics. Non-categorical variables were compared using the Student's *t*-test, 1-way analysis of variance with Bonferroni post hoc test. Generalized linear model with adjustment for age and gender was applied to evaluate the correlation between serum TGF-β1 concentrations and the Hughes' functional grading scale or INCAT disability scale at the time of sample collection. Each set of data was expressed as mean ± standard error. All *P* values were 2-tailed, and a $P < 0.05$ was considered significant. Receiver operating characteristic curve analysis was used to measure the ability of TGF-β1 concentration to predict the diagnosis of GBS.

3. Results

The demographic data of all groups were displayed in Table 1. Not surprisingly, the age of the patients with AD (75.7 ± 1.63 y) was significantly higher than the other groups ($P < 0.001$). IgG index in the patient with MS (1.02 ± 0.05) was significant higher compared with those with CIDP (0.60 ± 0.07, $P = 0.002$), GBS (0.69 ± 0.05, $P = 0.001$) and MFS (0.61 ± 0.15, $P = 0.026$). A panel of serum anti-ganglionside IgG auto-antibodies including anti-GM1, anti-GM2, anti-GM3, anti-GD1a, anti-GD1b, anti-GT1b and anti-GQ1b were measured for these patients. The results showed a number of CIDP patients had anti-GT1b (34.78%), anti-GM3 (30.43%) and anti-GD1b (26.09%). These auto-antibodies were present in fewer patients with GBS compared with those with CIDP, although the differences did not reach the statistical significance. Anti-GQ1b, thought as a diagnostic marker for MFS, was significantly increased (45.45%) in patients with MFS when compared with the other groups ($P = 0.008$–0.048). Paraneoplastic anti-neuronal antibodies including anti-AMPHIPHYSIN, anti-CV2, anti-Hu, anti-Ma2, anti-RECOVERIN, anti-Ri, anti-SOX1, anti-TITIN and anti-Yo were negative in all patients.

Activation of macrophage plays an important role in the peripheral neuroinflammatory diseases. By examining four macrophage-mediated inflammatory factors, IL-6, TGF-β1, VCAM-1 and VEGF, in the patients with CIDP, GBS, MFS, MS, and AD, as well as healthy controls, we found serum concentrations of TGF-β1 were significantly increased in GBS patients (35.94 ± 2.55 ng/ml, Fig. 1A) compared to those with CIDP (25.46 ± 1.40 ng/ml, $P < 0.001$), MFS (25.32 ± 2.31 ng/ml, $P = 0.010$), MS (21.35 ± 0.90 ng/ml, $P < 0.001$) and AD (22.92 ± 1.82 ng/ml, $P < 0.001$), as well as healthy controls (23.12 ± 1.67 ng/ml, $P < 0.001$). The area under ROC curve (AUC) for TGF-β1 was 0.78. None of the other three markers showed significant differences between the six groups (Fig. 1B–D). We analyzed the correlation between the serum concentrations of TGF-β1 and disease severity that was evaluated by scores of Hughes' functional grading scale (GBS and MFS) or INCAT disability scale (CIDP) at the time point of sample collection. The results showed a significant correlation between serum concentrations of TGF-β1 and Hughes' functional grading scale in the patients with GBS (β coefficient: 6.05 ± 1.26 ng/ml, $P < 0.001$, Fig. 2A and Table 2). On the other hand, such clinical correlation is absent in the patients with MFS and CIDP (Fig. 2B and C, Table 2). The CSF concentration of TGF-β1 was undetectable by the assay we used.

4. Discussion

Given that a peripheral nervous tissue sample from GBS patients is practically difficult to access, biomarkers in blood should be more feasible as an indicator for the disease severity as well as testing potential therapeutic strategies. In the present study, we demonstrated that serum TGF-β1 concentration was higher in the GBS patients when compared with MFS, CIDP and AD patients, as well as controls. Furthermore, a significant correlation between serum TGF-β1 concentrations and Hughes' functional grading scale scores was demonstrated in GBS