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Serum soluble Talin-1 levels are elevated in patients with multiple sclerosis, reflecting its disease activity

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ABSTRACT

Previously, we identified anti-Talin-1 antibodies in the serum of MS. In this case, we measured the serum soluble Talin-1 (sTalin-1) levels by enzyme-linked immunosorbent assay. The serum sTalin-1 levels were significantly higher in 40 patients with MS than in 43 normal controls and in the acute phase of disease than in the remission phase. Interestingly, serum sTalin-1 levels were associated with a sustained increase in disability after MS attack but not with serum anti-Talin-1 antibody levels. sTalin-1 may be a biomarker for the acute phase of MS and may be used for the short-term prognosis of MS.

1. Introduction

Multiple Sclerosis (MS) is an autoimmune, demyelinating disease, in which T cells are believed to play a pivotal role during pathogenesis. T cells are presumably primed in the peripheral tissues by antigens, undergo clonal expansion, pass thorough blood-brain barrier (BBB), become reactivated by antigens in the brain, and produce cytokines that lead to demyelination (Hemmer et al., 2002). However, antibodies, including oligoclonal IgG bands, can be frequently seen in the CSF of patients with MS. Treatment with monoclonal anti-CD20 antibody has shown high efficacy, especially for relapsing–remitting MS (Hauser et al., 2008). This accumulated evidence indicates that autoantibodies and B cells also play a substantial role in MS pathogenesis, at least in a subgroup of patients (Krumbholz et al., 2012).

Previously, we investigated serum autoantibodies in MS using serological analysis of recombinant cDNA expression libraries (SEREX) to identify autoantibodies (Muto et al., 2015). One of novel autoantibodies which we found is anti-talin1 antibody. Talin-1 is a cytoskeletal protein involved in actin filament assembly and cell migration. In this case, we identified anti-Talin-1 antibodies in the serum of a patient with MS and found that these antibodies were higher in patients with MS than in both healthy controls and patients with other diseases. These findings prompted us to speculate that soluble Talin-1 (sTalin-1) may be present in the serum of patients with MS and may play some role in disease pathogenesis. Also, sTalin-1 might have potential as a biomarker for MS. In this study, we measured serum sTalin-1 by enzyme-linked immunosorbent assay (ELISA) and looked for a correlation between its levels and clinical and laboratory parameters in patients with MS.

2. Patients and methods

2.1. Patients

Forty Japanese patients with relapsing–remitting MS, followed at the Chiba University Hospital, were involved in this study. Patients fulfilled the revised 2005 McDonald's criteria for MS (Polman et al., 2005). Forty-three healthy participants served as normal controls (NC).

The clinical condition of patients with MS was monitored with Kurtzke's disability status scale (EDSS). Using McDonald's criteria (Polman et al., 2005), clinical relapse was defined as current or historical patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, lasting at least 24 h, in the absence of fever or infection.

We reviewed sex, age, disease duration, duration from relapse onset to serum sampling (in days), and EDSS score (Kurtzke, 1983). We evaluated the change of EDSS with a focus on three time periods: pre-attack, during remission just before an attack; nadir, at clinical nadir during the attack and postattack, during remission after an attack but before the next attack. The difference in EDSS between the nadir and pre-attack, between postattack and preattack, and between postattack and nadir

Abbreviations: MS, multiple sclerosis; NC, normal control; BBB, blood-brain barrier; EDSS, Kurtzke's disability status scale.

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are expressed as $\Delta EDSS_{Nadir\text{-}Preattack}$, $\Delta EDSS_{Postattack\text{-}Preattack}$ and $\Delta EDSS_{Postattack\text{-}Nadir}$, respectively.

This study was approved by the Ethics Committee of the Chiba University School of Medicine. All participants provided written informed consent for their participation in this study.

2.2. CSF and serum sampling

Serum samples were obtained from patients with MS during the acute phase of a clinical attack within 2 months of the onset but before treatment. The median duration from attack onset to sample collection during the acute phase was 8.0 (interquartile range [IQR]: 13.5) days. In 13 of 40 patients, serum samples were also obtained during the remission phase after treatment and resolution of the attack and before the next attack. Median intervals from attack onset to sample collection during the remission phase was 77.0 (IQR 41.0) days. These 13 patients had been treated with intravenous (n = 11) or oral (n = 2) steroids after the attack had resolved but before the sampling during remission. All samples were stored at −80 °C until analysis. In 30 of 40 patients with MS, CSF samples were collected during the attack on the same day that serum samples were obtained. In addition, samples were evaluated for cell counts, protein levels, CSF/serum albumin ratio (Qalb), oligoclonal IgG bands (OCBs) positivity and IgG index. OCBs were determined by isoelectric focusing and were recorded as positive when they were detected only in the CSF and comprised at least two bands. IgG index was considered to be high when it was >0.75.

2.3. Measure of sTalin-1 in serum

Enzyme-linked immunosorbent assay for human Talin-1 was performed according to the manufacturer's instructions (MyBioSource, San Diego, California, USA). Briefly, 100 μl of a standard or sample were added per well, and the plates were incubated for 2 h at 37 °C. The liquid from each well was removed, and 100 μl of biotin-conjugated anti-human Talin-1 antibody was added to each well. After incubation for 1 h at 37 °C, the plates were washed and the liquid removed, and 100 μl of HRP-avidin was added to each well. After incubation for 1 h at 37 °C, repeat aspiration and washing were performed, and 90 μl of TMB (3,3′,5,5′-tetramethylbenzidine) substrate was added to each well. After additional incubation for 15–30 min at 37 °C and the addition of 50 μl of stop solution, the optical density was determined using a microplate reader at 450 nm and subtracting readings at 570 nm from those at 450 nm.

2.4. Serum anti-Talin-1 antibody levels

Of the 40 serum samples from patients with MS that were tested for sTalin-1 levels, and 38 of them had been measured serum anti-Talin-1 antibody levels in our previously study (Muto et al., 2015). We compared serum sTalin-1 levels and serum anti-Talin1 antibody levels.

2.5. Statistical analysis

Differences in values between the groups were analysed by the Mann–Whitney *U* test. The Wilcoxon signed-rank test was applied to compare paired parameters. Spearman's rank correlation coefficient was used to test associations. Tests were two-sided, and a value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS statistical package (version 11.0.1J, SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1. Clinical data for patients with MS

Table 1 provides the demographic, clinical and laboratory parameters for patients with MS and for NCs. Of the 40 patients, 31 were women and 9 were men. The median (IQR) age was 39.5 (15.5) years. The median (IQR) disease duration and EDSS score at serum sampling was 6.4 (11.2) years and 4.0 (2.5), respectively. OCBs were positive in 14 (40.0%) of 35 patients who were tested, and the IgG index was high in 15 (53.6%) of 28 patients who were tested. Treatments administered were interferon-β in 7 (17.5%) and fingolimod in 2 (5.0%). The remaining patients were not treated with any disease-modifying drug at the time of serum sampling. Of the 43 NCs, 28 were women and 15 were men. The median (IQR) age was 47.0 (13.5) years.

Table 1
Demographic, clinical and laboratory parameters of patients with multiple sclerosis and normal controls.

	MS	NC
Number	40	43
Men:women	9:31	15:28
Age, median (IQR), y	39.5 (15.5)	47.0 (13.5)
Disease duration, median (IQR), y	6.4 (11.2)	–
EDSS score, median (IQR)	4.0 (2.5)	–
Patients with positive OCBs, n (%)	14/35 (40.0%)	–
Patients with high IgG index[a] (%)	15/28 (53.6%)	–
Treatment		
At the time of serum sampling, n (%)	9/40 (22.5%)	–
Interferon-β	7/40 (17.5%)	–
Fingolimod	2/40 (5.0%)	–
None	31/40 (77.5%)	–

EDSS, Kurtzke's expanded disability status scale; IQR, interquartile range; NC, normal control; OCBs, oligoclonal IgG bands; –, not available.
[a] IgG index was considered to be high when it was >0.75.

3.2. Serum sTalin-1 levels

The median (IQR) serum sTalin-1 levels were 193.7 (97.0) and 158.6 (69.8) pg/ml in the MS and NC groups, respectively, significantly higher in the MS group compared to the NC group ($P = 0.0011$) (Fig. 1A). In addition, the serum sTalin-1 levels in patients with MS were significantly higher in the acute phase than in the remission phase ($P = 0.033$) (Fig. 1B). Serum positivity was defined as a sTalin-1 level more than two standard deviations above the mean value of 228.5 pg/ml in the 43 NC samples, so that 10 (25.0%) of 40 patients with MS had positive results. Comparison based on sTalin-1-positivity or –negativity (Supplementary Table 1) showed that patients who were serum sTalin-1-positive were less likely to have had at least one episode of optic neuritis. In addition, the serum sTalin-1 levels were lower in patients who had at least one episode of optic neuritis compared to those with no history of optic neuritis (median [IQR] 104.3 [86.6] pg/ml vs. 162.8 [106.3] pg/ml).

There were no significant differences between patients who were sTalin-1–positive and –negative for other demographic, clinical, or laboratory parameters including male-to-female ratio, age and disease duration at serum sampling, the ratio of patients who were treated with any disease modifying drug at serum sampling. Actually, the median (IQR) sTalin-1 levels in disease modifying drug-treated (n = 9) and –untreated patients (n = 31) were 227.6 (144.7) pg/ml, and 176.1 (92.9) pg/ml, respectively. The former was a little higher than the latter, but not significantly different.

We next compared the serum sTalin-1 levels in patients with demographic, clinical and laboratory parameters, including EDSS at the time the sera were sampled, $\Delta EDSS_{Nadir\text{-}Preattack}$, $\Delta EDSS_{Postattack\text{-}Preattack}$, and $\Delta EDSS_{Postattack\text{-}Nadir}$, CSF data (cell count, protein, albumin, Qalb), OCBs, IgG index and results of serum anti-Talin-1 antibody which were determined in our previous study (Muto et al., 2015). There was a positive correlation between serum sTalin-1 levels and $\Delta EDSS_{Postattack\text{-}Preattack}$ ($P = 0.036$) (Fig. 2A). Furthermore, there was a positive correlation between levels of serum sTalin-1 and CSF protein ($P = 0.041$) (Fig. 2B). The serum sTalin-1 levels were not