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Association between soluble L-selectin and anti-JCV antibodies in natalizumab-treated relapsing-remitting MS patients

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ABSTRACT

Objective: In relapsing-remitting MS (RRMS) patients treated with natalizumab, the low level of L-selectin-expressing CD4+ T cells has been associated with the risk of progressive multifocal leukoencephalopathy (PML). In this study, our aim was to correlate the levels of soluble L-selectin and the anti-JCV antibody index in the sera of RRMS patients treated with natalizumab.

Methods: This study included 99 subjects, including 44 RRMS patients treated with natalizumab, 30 with interferon beta (IFN-β) and 25 healthy controls. The levels of soluble L-selectin (sL-selectin) in sera were measured by ELISA, and the anti-JC Virus (JCV) antibody index was determined by the second–generation ELISA (STRATIFY JCV™ DxSelect™) assay.

Results: A significant correlation was found between the levels of sL-selectin and anti-JCV antibody indices in sera in the natalizumab-treated patients ($r=0.402$; $p=0.007$; $n=44$), but not in those treated with IFN-β. This correlation became even stronger in JCV seropositive patients treated with natalizumab for longer than 18 months ($r=0.529$; $p=0.043$; $n=15$).

Conclusion: The results support the hypothesis of sL-selectin being connected to the anti-JCV antibody index values and possibly cellular L-selectin. Measurement of serum sL-selectin should be evaluated further as a potential biomarker for predicting the risk of developing PML.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that is characterized by complex pathophysiological processes, including inflammation, demyelination, axonal loss and remyelination (Goldenberg, 2012). Natalizumab is a humanized monoclonal antibody and an α4 integrin (CD49d) antagonist that prevents the migration of peripheral leukocytes across the blood–brain barrier (BBB) (Hutchinson, 2007). It has been shown to reduce the relapse rate, decrease sustained disability, and reduce the number of new lesions on magnetic resonance imaging (MRI) (Miller et al., 2003; Polman et al., 2006). Despite its efficacy, long-term treatment (mostly more than 18 months) of natalizumab is associated with the substantial complication of developing progressive multifocal leukoencephalopathy (PML), a demyelinating lytic infection of the CNS caused by John Cunningham Virus (JCV) (Clifford et al., 2010). The precise mechanism of natalizumab-associated PML is still unclear, but it is suggested that PML occurs when immunosurveillance in the CNS is impaired (Mancuso et al., 2012). It has been proposed that blocking lymphocyte trafficking through the BBB during natalizumab therapy would decrease cell-mediated immunity, allowing the reactivation of the JC virus from latency (Berger and Houff, 2009). According to recent data, the relative incidence of natalizumab-associated PML is higher than 2/1000 patients (Buck and Hemmer, 2014). In addition to long-term natalizumab treatment, the prior use of immunosuppressants and the presence of anti-JCV antibodies have been established as contributing risk factors for developing PML (Bloomgren et al., 2012). Although anti-JCV antibodies are widely used for predicting the risk of developing PML, such antibodies are also measured in approximately 60–80% of healthy individuals. However, JCV reactivation and the development of PML are only rarely seen in healthy subjects (Ferenczy et al., 2012). Therefore, there is a high need for more

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sensitive biomarkers that would identify patients with a higher risk of developing natalizumab-associated PML.

Recently, a retrospective study showed that reduced levels of L-selectin-expressing CD4+ cells in blood were associated with a risk of developing natalizumab-associated PML (Schwab et al., 2013). Due to this observation, the authors proposed L-selectin as a possible biomarker for individual PML risk in MS patients. L-selectin (CD62L) is a cell adhesion molecule expressed on the surface of most circulating leukocytes, including T cells. It is also present as a functionally active soluble form in the blood (Raffler et al., 2005), which significantly increases during acute or chronic inflammation (Smalley and Ley, 2005). In this study, our aim was to evaluate whether the soluble form of L-selectin is associated with anti-JCV antibody indices in natalizumab-treated RRMS patients, which would suggest the potential of sL-selectin in the assessment of PML risk.

2. Patients and methods

2.1. Patients

This cross-sectional study included a total of 99 subjects of whom 44 RRMS patients were treated with natalizumab, 30 patients with IFN-β (21 patients with Rebif 22 μg and 9 with Rebif 44 μg) and 25 subjects were healthy controls (HC). MS patients were enrolled consecutively from four Finnish MS centers (Tampere, Helsinki, Seinäjoki, and Turku) between January 2012 and February 2013 based on their ongoing immunomodulatory therapy. The clinical characteristics of these patients are shown in Table 1. All patients underwent clinical and neurological examinations before blood sampling. The diagnosis of MS was based on the revised McDonald Criteria (Polman et al., 2005), and the diagnosis was definite. Neurological disability was evaluated by the expanded disability status scale (EDSS) score (Kurtzke, 1983). The study was approved by the Ethics Committee of Tampere University Hospital, and all subjects gave informed consent. The healthy individuals had no previous history of any neurological disorders or immune-mediated diseases.

2.2. Determination of sL-selectin /CD62L concentrations in serum

The collected blood was allowed to clot and was centrifuged for 15 min at 1500xg. Sera were separated from blood, aliquoted and stored at −80 °C until use. sL-selectin levels were determined by commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (#BBE4B; Quantikine, R&D Systems Europe Ltd, Abingdon, United Kingdom). Briefly, serum samples with 1:100 dilutions were mixed with a monoclonal antibody that is specific for human serum L-selectin, which was pre-coated on a 96-well microtiter plate. Horseradish peroxidase (HRP) conjugate, an enzyme-linked polyclonal antibody specific for human L-selectin, was then added. Color developed after TMB (Tetramethylbenzidine) substrate addition was stopped by adding hydrochloric acid (HCL) as a stop solution. The absorbances were measured at wavelength of 450 nm on a Multiskan MS version 4.0 spectrophotometer (Labsystems, Helsinki, Finland). The intra- and inter-assay coefficients of variation for the sL-selectin assay was 4.1% and 7.1%, respectively. The minimum detection limit for sL-selectin assay was 0.3 ng/mL.

2.3. Determination of the anti-JCV antibody index

A confirmatory second–generation ELISA (STRATIFY JCV™ DxSelect) was used to test sera for anti-JCV antibodies at the Unilabs, Denmark (Lee et al., 2013). A screen index value of less than 0.2 was considered anti-JCV antibody negative and of greater than 0.4 as anti-JCV antibody positive. The samples with a screen index between 0.2 and 0.4 were evaluated with a supplementary confirmatory test, and results greater than 45% were classified as anti-JCV antibody positive (Lee et al., 2013).

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A non-parametric, two-tailed Mann–Whitney U test was used to compare the differences between the clinical parameters and levels of sL-selectin in different groups. Spearman's correlation coefficient was used to analyze the correlation between the sL-selectin levels and anti-JCV antibody index. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical data

Natalizumab-treated patients had a longer disease duration

Table 1
Clinical characteristics of MS patients and healthy controls.

Characteristics	Natalizumab	IFN-β	HC	p-value
	$n=44$	$n=30$	$n=25$	
Sex (F/M)[a]	34/10	21/9	19/6	
Age (years)[b]	38.2 ± 7.8 (23-52)	35.5 ± 9.9 (20-53)	33.2 ± 11.0 (22–60)	NS
Disease duration from diagnosis (years)[b]	9.1 ± 5.3 (1.8–22.4)	4.6 ± 5.4 (0.2-18.1)	–	$p<0.001$
EDSS[b]	2.7 ± 1.9 (0–6.5)	1.4 ± 1.6 (0-6.0)	–	$p=0.002$
Number of relapses[b,c]	1.9 ± 1.0 (1-4)	–	–	–
Duration of treatment (years)[b]	2.8 ± 1.5 (0.4–5.8)	2.5 ± 2.8 (0–13.1)	–	NS
Anti-JCV Ab index [d]	0.3 (0.1–3.1)	0.3 (0.1–2.9)	–	NS
Anti-JCV Ab seropositivity[a]	21 (48%)	13 (43%)	–	NS
JCV-positive Ab index[d,e]	1.1 (0.3–3.1)	1.9 (0.7–2.9)	–	NS

IFN-β – interferon-β, **HC –** healthy controls, **EDSS** – expanded disability status scale, **JCV –** John Cunningham virus, **NS –** not significant

[a] Number of patients.
[b] Mean ± SD (range).
[c] Two years before starting Natalizumab.
[d] Median (range).
[e] Anti-JCV antibody index of seropositive patients only.