Mult Scler OnlineFirst, published on May 18, 2010 as doi:10.1177/1352458510368051

Short Report

Multiple Sclerosis

Multiple Sclerosis
0(00) 1–5

DOI: 10.1177/1352458510368051
msj.sagepub.com
SAGE

Epstein–Barr virus-specific antibody response in cerebrospinal fluid and serum of patients with multiple sclerosis

Massimiliano Castellazzi[1], Carmine Tamborino[1], Alice Cani[1], Elena Negri[1], Eleonora Baldi[2], Silva Seraceni[3], Maria Rosaria Tola[2], Enrico Granieri[1], Carlo Contini[3] and Enrico Fainardi[4]

Abstract

Cerebrospinal fluid and serum levels and intrathecal synthesis of anti-Epstein–Barr virus (EBV) IgG were measured by enzyme-linked immunosorbent assay in 80 relapsing–remitting multiple sclerosis patients grouped according to clinical and magnetic resonance imaging (MRI) evidence of disease activity. Eighty patients with other inflammatory neurological disorders (OIND) and 80 patients with non-inflammatory neurological disorders (NIND) served as neurological controls. Cerebrospinal fluid concentrations were higher in OIND than in multiple sclerosis ($p < 0.0001$) and NIND ($p < 0.01$) for anti-viral-capsid-antigen (anti-VCA) IgG, in multiple sclerosis than in NIND ($p < 0.01$) and in OIND than in NIND ($p < 0.05$) for anti-EBV nuclear antigen-1 (EBNA-1) IgG. Serum levels were more elevated in OIND than in multiple sclerosis ($p < 0.05$) and in MRI inactive than in MRI active multiple sclerosis ($p < 0.0001$) for anti-VCA IgG, and in multiple sclerosis than in OIND and NIND ($p < 0.01$) for anti-EBNA-1 IgG. Serum titres of anti-VCA and anti-EBNA-1 IgG were also positively ($p < 0.05$) and inversely ($p < 0.001$) correlated, respectively, with the Expanded Disability Status Scale. An intrathecal IgG production of anti-VCA and anti-EBNA-1 IgG, as indicated by Antibody Index, was present only in a limited number of multiple sclerosis patients and controls (range from 1.3 to 6.3%). These findings do not support a direct pathogenetic role of EBV-targeted humoral immune response in multiple sclerosis.

Keywords

cerebrospinal fluid, Epstein–Barr virus, intrathecal synthesis, multiple sclerosis

Date received: 21st December 2009; revised: 1st February 2010; accepted: 5th March 2010

Introduction

Epstein–Barr virus (EBV) has recently received renewed interest as a potential causative infectious agent in multiple sclerosis (MS)[1] since EBV-infected B cells expressing both latent and lytic viral proteins have been found in post-mortem brain tissue from MS patients.[2] However, the significance of EBV-specific humoral immune response in MS still remains to be elucidated because the detection of EBV-infected B cells in MS brain lesions was reported in some recent publications[3–5] as having failed. Elevated serum levels of EBV-specific antibodies have been observed more frequently in MS than in controls and increase the risk of developing MS.[1] High serum concentrations of IgG directed to EBV nuclear antigen-1 (EBNA-1) and viral-capsid-antigen (VCA), considered as latent and lytic markers, have been associated with magnetic resonance imaging (MRI) evidence of MS disease activity[6] and cortical atrophy,[7] respectively. Serum anti-EBV IgG levels were also elevated in patients with clinically isolated syndromes in whom they were able to predict conversion to MS.[8] In addition, EBV-specific oligoclonal IgG predominantly binding EBNA-1 has been isolated from the cerebrospinal fluid (CSF)

[1]Section of Neurology, Department of Medical and Surgical Sciences of Communication and Behaviour, University of Ferrara, Ferrara, Italy.
[2]Neurology Unit, Department of Neurosciences and Rehabilitation, Azienda Ospedaliera-Universitaria, Ferrara, Italy.
[3]Section of Infectious Diseases, Department of Clinical and Experimental Medicine, University of Ferrara, Ferrara, Italy.
[4]Neuroradiology Unit, Department of Neurosciences and Rehabilitation, Azienda Ospedaliera-Universitaria, Ferrara, Italy.

Corresponding author:
Massimiliano Castellazzi BS, Section of Neurology, Department of Medical and Surgical Sciences of Communication and Behaviour, University of Ferrara, Corso della Giovecca 203, Ferrara I-44100, Italy.
Email: massimiliano.castellazzi@unife.it

of MS patients.[9] Conversely, while CSF-restricted anti-EBV oligoclonal IgG has been detected in a variable proportion of MS patients,[2,10] a quantitative intrathecal synthesis of anti-EBV IgG was present only in a small subset of MS patients.[11] Considering these observations, the aim of our study was to investigate CSF and serum levels and the presence of an intrathecal synthesis of anti-EBV IgG in MS and controls.

Patients and methods

This study included 80 consecutive untreated patients (55 females and 25 males; mean age = 37.8 ± 11.0 years) with relapsing–remitting (RR) definite MS according to the McDonald criteria.[12] All patients were imaged with a 1.5-Tesla MRI unit within 48 hours after sampling. Evidence of a relapse at admission and lesions showing Gd enhancement on T1-weighted scans were considered as clinical and MRI disease activity, respectively. Disease severity was scored at the time of sample collection using the Expanded Disability Status Scale (EDSS). The duration of the disease was expressed in months. At entry none of the patients had fever or other symptoms or signs of acute infections. Moreover, at the time of sample collection none of the patients had received any potential disease-modifying therapies during the 6 months before the study. Eighty patients with other inflammatory neurological disorders (OIND) and 80 patients with non-inflammatory neurological disorders (NIND) were used as neurological controls. The OIND group (53 females and 27 males, mean age = 39.3 ± 10.3 years) included 14 patients with chronic inflammatory demyelinating polyneuropathy, 13 with acute inflammatory demyelinating polyneuropathy, 9 with bacterial meningitis, 9 with HIV encephalopathy, 8 with herpes simplex virus-1 encephalitis, 6 with acute disseminated encephalomyelitis, 6 with intracerebral abscess, 5 with varicella-zoster virus encephalitis, 5 with neurolupus, 3 with neuroSjogren and 2 with neuroBehçet. The NIND group (52 females and 28 males, mean age = 38.9 ± 10.5 years) consisted of 15 patients with headache, 11 with migraine, 9 with transient ischaemic attack, 7 with epilepsy, 7 with cervical spondylosis, 6 with vascular dementia, 6 with mild cognitive impairment, 5 with Alzheimer's disease, 5 with hereditary ataxia, 3 with amyotrophic lateral sclerosis, 2 with compression neuropathy, 2 with paresthesias, 1 with confusion and 1 with vascular myelopathy. The study design was approved by the Local Committee for Medical Ethics in Research.

Paired CSF and serum samples prospectively collected from MS, OIND and NIND patients were obtained for purposes of diagnosis and measured under exactly the same conditions. CSF and serum albumin and IgG levels were measured by immunochemical nephelometry.[13] Routine CSF analysis was performed according to the currently accepted recommendations.[14] CSF and serum levels of anti-EBNA-1 and anti-VCA IgG were measured by enzyme-linked immunosorbent assay (ELISA) (Novagnost EBV-EBNA IgG and EBV-VCA IgG). In accordance with Reiber and Lange,[15] a reference curve was generated in each assay using six serial dilutions of pooled high positive serum samples ranging between 0.1 and 2.0 OD. CSF and corresponding serum were two-fold diluted with a range of 1:2–1:6 and 1:100–1:1200, respectively. Anti-EBNA-1 and anti-VCA IgG levels of each sample were expressed in arbitrary units obtained by multiplying concentrations extrapolated from the standard curve to the dilution factor. Within-assay and between-assay precisions were determined after 10 repeated measured into the same plate and by repetition of the same sample in 10 consecutive plates, respectively. The intra-assay coefficient of variations (CV), calculated by the formula CV = standard deviation/mean × 100, was 6.1% for anti-EBNA-1 IgG and 8.5% for anti-VCA IgG, whereas the inter-assay CV was 6.3% for anti-EBNA-1 IgG and 12.8% for anti-VCA IgG. Production of anti-EBV IgG was determined by Antibody Index (AI), which was calculated following the original formula based on the appropriate corrections to discriminate between blood-derived and brain-derived CSF antibody fraction.[15] EBV-specific intrathecal IgG synthesis was assumed for values of AI $\geq$ 1.5. After checking data for normality by using the Kolmogorov–Smirnov test, the Kruskal–Wallis test, followed by the Mann–Whitney U test, chi-square test (χ^2), and the Spearman rank correlation coefficient test were used when appropriate. Bonferroni correction was utilized for multiple comparisons.

Results

Clinical, radiological and CSF findings from MS patients are listed in Table 1. As shown in Figure1, CSF mean levels of anti-VCA IgG were more elevated in OIND than in MS ($p < 0.0001$) and NIND ($p < 0.01$) (panel A), whereas serum mean concentrations of anti-VCA IgG were higher in OIND than in MS ($p < 0.05$) and in MRI inactive than in MRI active MS ($p < 0.0001$) (panel B). On the other hand, while CSF mean levels of anti-EBNA-1 IgG were greater in MS than in NIND ($p < 0.01$) and in OIND than in NIND ($p < 0.05$) (panel D), serum mean titres of anti-EBNA-1 IgG were more increased in MS than in OIND and NIND ($p < 0.01$) (panel E). In addition, serum levels of anti-VCA IgG and anti-EBNA-1 IgG correlated positively ($p < 0.05$) and inversely ($p < 0.001$), respectively, with EDSS (panels C and F). No other significant differences

Table 1. Clinical, radiological and CSF characteristics in 80 patients with relapsing–remitting multiple sclerosis

Disease duration, months: mean ± SD	30.0 ± 42.0
Disease severity, EDSS: mean ± SD	2.0 ± 1.4
CA MS: *n*/total (%)	63/80 (78.7%)
CS MS: *n*/total (%)	17/80 (21.3%)
Gd+ MS: *n*/total (%)	30/80 (37.5%)
Gd− MS: *n*/total (%)	50/80 (62.5%)
Blood-CSF-barrier dysfunction, QAlb: positive/total (%)	13/80 (16.3%)
Intrathecal IgG synthesis, IgG index: positive/total (%)	53/80 (66.3%)
Intrathecal IgG synthesis, intrathecal fraction: positive/total (%)	54/80 (67.5 %)
Oligoclonal bands, IEF: positive/total (%)	73/80 (91.3%)

EDSS, Expanded Disability Status Scale; CA, clinically active (presence of relapse at entry); CS, clinically stable (absence of relapse at entry); Gd+, MRI appearance of gadolinium-enhanced lesions; Gd−, no MRI evidence of gadolinium-enhanced lesions; Qalb, CSF/serum albumin quotient; IEF, isoelectric focusing followed by IgG-specific immunoblotting.

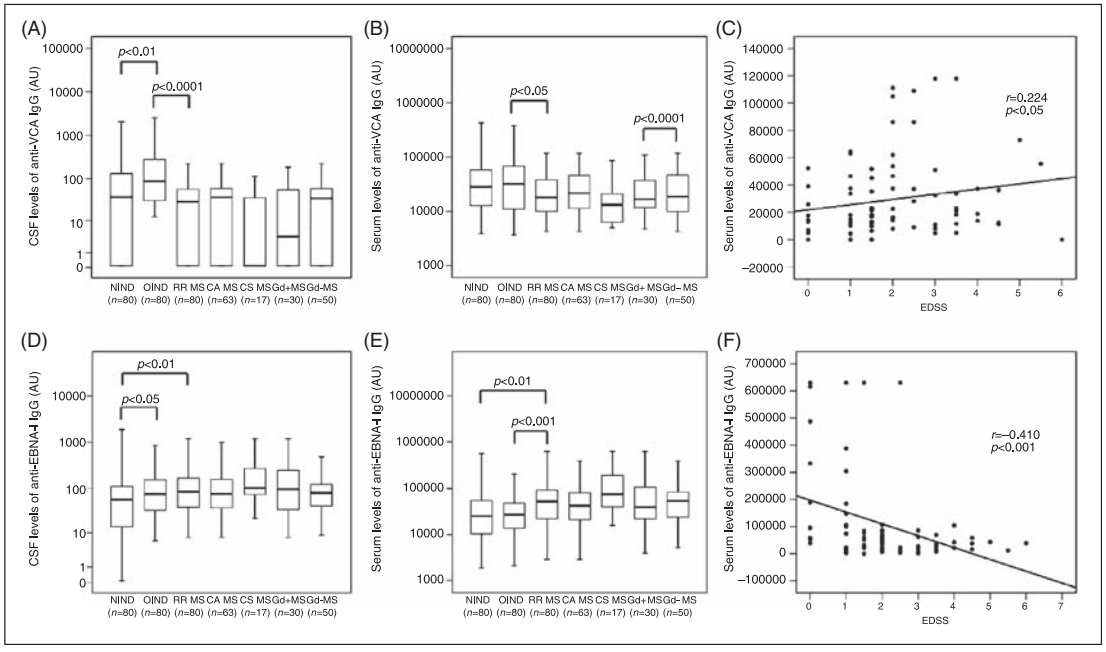

Figure 1. CSF and serum levels of anti-VCA and anti-EBNA-1 IgG from subjects with non-inflammatory neurological disorders (NIND), other inflammatory neurological disorders (OIND) and from patients with multiple sclerosis (MS), considered as a whole and classified according to clinical course and evidence of clinical and magnetic resonance imaging (MRI) disease activity, together with relationships between serum levels of anti-VCA and anti-EBNA-1 IgG and Expanded Disability Status Scale (EDSS). CSF and serum levels of anti-VCA IgG were different among MS, OIND and NIND (Kruskal–Wallis; $p < 0.0001$ and $p < 0.05$, respectively). CSF values were more elevated in OIND than in MS (Mann–Whitney; $p < 0.0001$) and NIND (Mann–Whitney; $p < 0.01$) (panel A), whereas serum titres were more increased in OIND than in MS (Mann–Whitney; $p < 0.05$) and in MRI inactive than in MRI active MS (Mann–Whitney; $p < 0.0001$) (panel B). There was a trend towards a positive correlation (Spearman; $p < 0.05$) between serum anti-VCA IgG concentrations (y-axis) and EDSS (x-axis) (panel C). CSF and serum levels of anti-EBNA-1 IgG were different among MS, OIND and NIND (Kruskal–Wallis; $p < 0.01$ and $p < 0.001$, respectively). CSF concentrations were higher in MS than in NIND (Mann–Whitney; $p < 0.01$) and in OIND than in NIND (Mann–Whitney; $p < 0.05$) (panel D), whereas serum amounts were greater in MS than in OIND and NIND (Mann–Whitney; $p < 0.01$) (panel E). There was a trend towards an inverse correlation (Spearman; $p < 0.001$) between serum anti-EBNA-1 IgG levels (y-axis) and EDSS (x-axis) (panel F). RR: relapsing–remitting, SP: secondary progressive, PP: primary progressive, CA: clinically active (presence of relapse at entry), CS: clinically stable (absence of relapse at entry), Gd+: MRI appearance of gadolinium-enhanced lesions, Gd−: no MRI evidence of gadolinium-enhanced lesions. The boundaries of the boxes represent the 25th–75th quartile. The line within the box indicates the median. The vertical lines above and below the box correspond to the highest and lowest values, excluding outliers.

were found for CSF and serum levels of anti-VCA IgG and anti-EBNA-1 IgG among the groups evaluated. In MS patients, we did not observe further definite relationships between duration and severity of the disease and concentrations of anti-VCA IgG and anti-EBNA-1 IgG in CSF and serum. AI values suggestive of an intrathecal synthesis of anti-VCA and anti-EBNA-1 IgG were present in a small proportion of MS (2/80; 2.5% and 5/80; 6.3%, respectively), OIND (both 3/80; 3.8%, respectively) and NIND (1/80; 1.3% and 2/80; 2.5%, respectively), without any statistical differences among the groups studied.

Discussion

In agreement with a recent publication,[11] our results argue against a possible implication of EBV-targeted humoral immune response in MS pathogenesis since the presence of an intrathecal production of anti-EBV IgG in only a small proportion of MS patients and controls indicates that EBV-specific intrathecally produced antibodies are a part of a polyspecific humoral reactivity promoted by overactive MS chronic brain inflammation. Surprisingly, after results reported by others,[7] we found that CSF and serum levels of anti-VCA IgG were lower in MS than in several inflammatory neurological conditions with high serum concentrations of anti-VCA IgG when disease inflammatory activity resolves. Moreover, we detected more elevated CSF and serum values of anti-EBNA-1 IgG in MS than in controls. These data were substantially in line with a recent publication,[8] but did not confirm the previously reported utility of serum anti-EBNA-1 IgG concentrations as a biomarker for MS disease activity.[6] Nevertheless, the lack of spinal cord MRI examinations in our study could affect this finding since some small active lesions could be missed, leading to potentially inappropriate allocation of patients to the MRI inactive group. On the other hand, we identified, for the first time, a trend towards reciprocal correlations between anti-VCA IgG and anti-EBNA-1 serum levels and disease severity, which, however, were quite weak in magnitude. In addition, the over-time correlation between fluctuations in CSF and serum levels of anti-EBNA-1 and anti-VCA IgG and EDSS was not investigated in this study, making the interpretation of our data increasingly difficult. The divergences are probably due to methodological differences in determination techniques and patient selection, underlining the need of future standardization. In fact, in previous studies[6,7] the measurements of anti-EBV antibodies were performed by chemiluminescent immunoassays in MS patients grouped according to the different clinical courses and not in controls, whereas in our investigation anti-EBV IgG was assessed by ELISA methods in MS patients suffering only from the RR form and also in controls. Collectively, these observations suggest that MS could be characterized by a systemic EBV latent infection that seems to shift towards a lytic profile, likely reflecting virus reactivation, during the remission and progression of the disease. However, our findings do not exclude the hypothesis that a dysregulated EBV infection of B cells can play a role in MS autoimmunity.[2]

Acknowledgements

This work has been supported by FISM – Fondazione Italiana Sclerosi Multipla – Cod. 2008/R/12 and by Research Program Regione Emilia Romagna – University 2007-2009 (Innovative Research), entitled 'Regional Nerwork for Implementing a Biological Bank to Identify Biological Markers of Disease Activity Related to Clinical Variables in Multiple Sclerosis'. The authors thank Dr Elizabeth Jenkins for helpful corrections to the manuscript.

References

1. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann Neurol* 2007; 61: 288–299.
2. Serafini B, Rosicarelli B, Franciotta D, et al. Dysregulated Epstein–Barr virus infection in the multiple sclerosis brain. *J Exp Med* 2007; 204: 2899–2912.
3. Opsahl ML, Kennedy PGE. An attempt to investigate the presence of Epstein Barr virus in multiple sclerosis and normal control brain tissue. *J Neurol* 2007; 254: 425–430.
4. Willis SN, Stadelmann C, Rodig SJ, et al. Epstein–Barr virus infection is not a characteristic feature of multiple sclerosis brain. *Brain* 2009; 132: 3318–3328.
5. Peferoen LAN, Lamers F, Lodder LNR, et al. Epstein–Barr virus infection is not a characteristic feature in the central nervous system in established multiple sclerosis. *Brain* 2009 16 Nov; (Advance Access) DOI: 10.1093/brain/awp296.
6. Farrell RA, Antony D, Wall GR, et al. Humoral immune response to EBV in multiple sclerosis is associated with disease activity on MRI. *Neurology* 2009; 73: 32–38.
7. Zivadinov R, Zorzon M, Wienstock-Guttmann B, et al. Epstein–Barr virus is associated with grey matter atrophy in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009; 80: 620–625.
8. Lünemann JD, Tintoré M, Messmer B, et al. Elevated EBNA1 immune responses predict conversion to multiple sclerosis. *Ann Neurol* 2009 13 Oct; (accepted article) DOI: 10.1002/ana. 21886.
9. Cepok S, Zhou D, Srivastava R, et al. Identification of Epstein–Barr virus proteins as putative targets of the immune response in multiple sclerosis. *J Clin Invest* 2005; 115: 1352–1360.
10. Rand KH, Houck H, Denslow ND, Heilman KM. Epstein–Barr virus nuclear antigen-1 (EBNA-1) associated oligoclonal bands in patients with multiple sclerosis. *J Neuroimmunol* 2000; 173: 32–39.

11. Pohl D, Rostasy K, Jacobi C, et al. Intrathecal antibody production against Epstein–Barr and other neurotropic viruses in pediatric and adult onset multiple sclerosis. *J Neurol* 2009 28 Aug; (Epub ahead of print) DOI 10.10007/s00415-009-5296-y.
12. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. *Ann Neurol* 2001; 50: 121–127.
13. Salden HJM, Bas BM, Hermas JT, Janson PC. Analytical performance of the three commercially available nephelometers compared quantifying protein in serum and cerebrospinal fluid. *Clin Chem* 1988; 34: 1594–1596.
14. Franciotta D, Avolio C, Capello E, Lolli F, participating members AINI. Consensus recommendations of the Italian Association for Neuroimmunology for immunochemical cerebrospinal fluid examination. *J Neurol Sci* 2005; 237: 5–11.
15. Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain. *Clin Chem* 1991; 37: 1153–1160.