

SHORT REPORT

Altered antibody pattern to Epstein-Barr virus but not to other herpesviruses in multiple sclerosis: a population based case-control study from western Norway

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Abstract

Objective—The prevalence of anti-EBV antibodies was studied in a group of 144 patients with multiple sclerosis and 170 age, sex, and area matched controls from the county of Hordaland, western Norway. The prevalence of three other herpesviruses, herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV), were also included.

Methods—Antibodies to various virus antigens were determined by enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) in serum samples from 144 patients with multiple sclerosis and 170 controls.

Results—All of the 144 patients with multiple sclerosis had IgG antibodies to EBV compared with 162 of 170 controls ($p=0.008$). The frequency of IgG antibodies to EBV capsid antigen (VCA), nuclear antigen (EBNA), and early antigen (EA) was significantly higher in patients with multiple sclerosis compared with the controls ($p<0.000001$, $p=0.01$, and $p<0.0001$ respectively). The presence of antibodies was independent of the initial course of the disease and the disease activity at the time of blood sampling. The prevalence of IgG antibodies to HSV, CMV, and VZV did not differ between cases and controls.

Conclusion—The results suggest a role for EBV in the aetiology of multiple sclerosis.

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Keywords: multiple sclerosis; Epstein-Barr virus; herpesvirus; antibodies

Multiple sclerosis is an inflammatory demyelinating disorder of the CNS. Although the aetiology is unknown, epidemiological studies suggest that infections during late childhood or early adolescence may predispose genetically susceptible people to later development of the disease. Numerous infectious agents, both viral and bacterial, have been suggested as being involved in the aetiology of multiple sclerosis,

but so far no agent has been consistently associated with the disease.¹

Viruses that can establish persistent or latent infections in the CNS or the immune system are attractive candidates as aetiological agents in a chronic neurological disorder such as multiple sclerosis. Epstein-Barr virus (EBV) has this ability,² and among various neurological complications, demyelinating disease has been found after primary EBV infection.^{3,4}

Epidemiological evidence for an association between EBV and multiple sclerosis has also been reported. Increased risk of multiple sclerosis has been found in people with a history of infectious mononucleosis.⁵⁻⁸ The peak incidence for infectious mononucleosis is at the age of 15-25 years, with a mean age at 18 for males and 16 for females⁹ which is within the critical period of exposure to an exogenous factor suggested by several migration studies.¹⁰⁻¹² Further, some reports have shown a higher prevalence (99%-100%) of anti-EBV antibodies in patients with multiple sclerosis than in controls (84-95%).¹³⁻¹⁷ Others have not been able to confirm these findings.^{18,19} Differences may reflect selection of patients or small numbers. None of the studies were population based. We have previously reported a raised EBV related autoimmune response in patients with multiple sclerosis²⁰ and clustering of places of residence of patients with multiple sclerosis at the ages of 13-20 years,²¹ which coincides with the ages of high incidence of infectious mononucleosis.⁹ In the present report, we have studied the seroprevalence of anti-EBV antibodies in the same group of patients with multiple sclerosis and controls from the county of Hordaland. The seroprevalence of three other herpesviruses, herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV), were also included.

Materials and methods

PATIENTS

All patients with multiple sclerosis within the county of Hordaland, western Norway and with clinical onset within that county, during the period 1976 to 1986 and diagnosed before

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Antibodies to herpesviruses among patients with multiple sclerosis and controls

Virus/type of antibody	MS positive (%)		Controls positive (%)		Test of difference between groups	
	n=144		n=170		O/R	p Value
EBV VCA-IgG	141	(97.9)	138	(81.2)	10.90	<0.000001
VCA-IgM	2	(1.4)	1*	(0.7)	1.99	0.57†
EBNA-IgG	143	(99.3)	160	(94.1)	8.94	0.013
EA-IgG	99	(68.8)	79	(46.5)	2.53	<0.0001
Total‡	144	(100)	162	(95.3)	—	0.0084
HSV-IgG	117	(81.3)	148	(87.1)	0.64	0.16
VZV-IgG	141	(97.9)	167	(98.2)	0.84	0.84
CMV-IgG	91	(63.2)	117	(68.8)	0.78	0.29

*Test performed in 142 subjects.

†Fisher's exact test.

‡Tested positive to one or more EBV antigens.

MS= multiple sclerosis; O/R= odds ratio; EBV= Epstein-Barr virus; VCA= viral capsid antigen; EBNA= Epstein-Barr virus nuclear antigen; EA= early antigen; HSV= herpes simplex virus; VZV= varicella zoster virus; CMV= cytomegalovirus.

1 January 1987, were selected for the study. The total of 144 patients comprised 61 men and 83 women, aged 17–66 years (mean age 39.2 years). According to the criteria of McAlpine,²² 130 (90.3%) patients were classified as having definite multiple sclerosis, 10 (6.9%) as having probable multiple sclerosis, and four (2.8%) as having possible multiple sclerosis. Mean duration of disease was 6.9 years, range 1–11 years, and the initial course of the disease was relapsing-remitting in 117 (81.2%) and primary progressive in 27 (18.8%). Serum samples were collected in 1988 and 15 (10.3%) of the patients were in acute relapse at the time of blood sampling.

CONTROLS

Controls comprised 170 patients admitted to hospital with traumatic fractures, traumatic rupture of ligaments, or minor gynaecological or plastic surgery disorders. There were 77 men and 93 women, aged 18–77 years (mean age 40.0 years) and they were category matched to the cases according to age (five year age groups), sex, and area of residence (inland, coastal, and urban area) that gave a similar distribution of cases and controls for these variables. Blood sampling was performed at the same time as for the patients.

ANTIBODY ANALYSIS

IgG and IgM antibodies to EBV viral capsid antigen (VCA) were determined by enzyme linked immunosorbent assay (ELISA) (Dupont, N Billerica, MA, USA) and indirect immunofluorescence (IIF) (Organon Teknika, Durham, NC, USA). IgG antibodies to EBV nuclear antigen (EBNA) and early antigen (EA) were measured by IIF (Organon Teknika, Durham, NC, USA). IgG antibodies to HSV, VZV, and CMV were determined by ELISA (Enzygnost, Behringwerke, Marburg, Germany). All assays included negative and positive controls.

STATISTICAL ANALYSIS

Comparisons were analysed by the χ^2 method, or Fisher's exact test for small numbers. The odds ratio was used to estimate the risk of being a case given the presence of exposure. In addition, a multivariate logistic regression analysis was performed with sex and age as covariables

to test for any residual effects of these variables. Significance was set at $p < 0.05$.

Results

The table shows that anti-VCA IgG antibodies were detected in 97.9% of the patients with multiple sclerosis and 81.2% of the controls ($p < 0.000001$). IgM antibodies to VCA were present in 1.4% of the patients with multiple sclerosis compared with 0.7% of the controls ($p = 0.57$). IgG antibodies to EA were present in 68.8% of the patients with multiple sclerosis compared with 46.5% of the controls ($p = 0.00007$).

Anti-EA antibodies were present in 71.8% of the patients with relapsing-remitting disease compared with 55.6% of the patients with primary progressive disease ($p = 0.10$) and 10.7% of the anti-EA positive patients with relapsing-remitting disease were in acute relapse at the time of blood sampling compared with 18.2% of the anti-EA negative patients with relapsing-remitting disease ($p = 0.28$). Thus the anti-EA response was independent of the initial course of the disease and the disease activity at the time of blood sampling. IgG antibodies to EBNA were present in 99.3% of the patients with multiple sclerosis and 94.1% of the controls ($p = 0.013$). Only one patient was anti-EBNA negative, but this patient tested positive for anti-VCA IgG antibodies. Thus all patients with multiple sclerosis (100%) were anti-EBNA or anti-VCA IgG positive compared with 162 (95.3%) of the controls ($p = 0.008$).

The table shows that patients and controls did not differ in the prevalence of antibodies to other herpesviruses (HSV, VZV, and CMV).

The controls were category matched to the cases according to age and sex. However, to test any residual effect of these variables a multivariate logistic regression analysis was performed with sex and age as covariables; these analyses presented only minor changes in odds ratios and p values.

Discussion

The presence of serum IgG antibodies to EBV EBNA, or VCA, or both, indicating a history of EBV infection, were present in all the patients with multiple sclerosis and were significantly more frequent than in the control population, confirming the results of previous studies.^{13–17} Further, a co-twin study has reported a higher frequency of anti-EBV IgG antibodies in the twins with multiple sclerosis compared with their healthy co-twins.²³ Coyle *et al*¹⁹ have reported a higher frequency of anti-EBV antibodies in patients with multiple sclerosis (13 of 22) compared with controls (three of 12) although the numbers were small. The sensitivity of the test in this study was probably low because the prevalence of anti-EBV antibodies in the control group was much lower than expected.^{13–17} Compston *et al*¹⁸ reported a lower prevalence of anti-EBV antibodies in the patient group (76%) and controls (73%). However, the patient group included patients with optic neuritis and isolated demyelinating lesions in addition to patients with clinically definite multiple sclerosis. Therefore this study

is not readily comparable with the other studies with only patients with multiple sclerosis included. In addition Compston *et al* used other methods in their analysis (IIF) compared with the present study (ELISA) that could contribute to the different results.

The presence of anti-EA antibodies indicates acute or chronic active EBV infection and onset of viral replication.² IgG Antibodies to EA were significantly more frequent among patients with multiple sclerosis than normal controls, compatible with the results reported by Sumaya *et al*.¹⁶ In the present study, two patients with multiple sclerosis and one control were anti-VCA IgM positive without clinical evidence of acute EBV infection. Further, the anti-EA response was independent of the initial course of the disease and the disease activity at the time of blood sampling. Taken together the results suggest that the patients with multiple sclerosis often have a chronic active form of the EBV infection, independent of the clinical course and activity of the disease.

No differences in antibody responses to other herpesviruses were found between the patients and controls. The prevalence of anti-HSV IgG antibodies was within the range of previous reports.^{14 19 24} The prevalence of anti-VZV IgG antibodies was higher than in most other studies, but confirms previous reports of no difference between the two groups.^{14 18 19} Two previous studies of CMV IgG antibodies have shown higher frequency among the patients with multiple sclerosis than among controls, and^{14 19} one study found no difference between the two groups,¹⁸ compatible with the present study.

There may be several explanations for the EBV antibody pattern in the present study. It could reflect an altered immune response in multiple sclerosis or implicate EBV as an aetiological factor of the disease. An altered immune response to EBV in multiple sclerosis may reflect reduced suppressor activity, which has been described in such patients.²⁵ In addition, Craig *et al*²⁶ have reported reduced T lymphocyte mediated control of B lymphocytes infected with EBV in patients with multiple sclerosis. This may lead to frequent reactivation of the latent EBV infection, which could explain the higher prevalence of EA antibodies in the patients with multiple sclerosis. Alternatively, EBV could induce demyelination in multiple sclerosis by local infection of oligodendrocytes or other glial cells leading to release of factors causing demyelination. However, there is no evidence for such a local EBV infection in the CNS of patients with multiple sclerosis.²⁷ Demyelination by cross reacting autoantibodies is another theory. Our recent report of anti EBNA-1 antibodies cross reacting with antigens in neuroglial cells²¹ is compatible with this hypothesis. Furthermore, activation of autoreactive T lymphocytes by EBV supports the hypothesis that multiple sclerosis can be initiated by a ubiquitous infection in late childhood or early adolescence that induces an immunological response in genetically predisposed people. Such an activation could occur by EBV peptides that have

sequence similarity with myelin epitopes (molecular mimicry) or by EBV superantigens. To our knowledge, there are no reports of EBV peptides as superantigens in multiple sclerosis, but there are findings that can support the theory of molecular mimicry. Two studies have reported peptide sequence identities between myelin basic protein and EBV, but these were computer analyses and did not take into account secondary and tertiary structures which are of importance in the immune response.^{28 29} Of more interest is the report of Wucherpfennig *et al*³⁰ that EBV mimicry peptides can activate myelin basic protein specific T cell clones.

The hypothesis of cross reacting autoantibodies and molecular mimicry is attractive, but substantial evidence that such mechanisms exist in multiple sclerosis has not yet been presented. Nevertheless, with reference to the anti-EBNA antibody results in the present study, it is of interest that Bray *et al*²⁹ reported a higher frequency of anti-EBNA-1 antibodies in the CSF of patients with multiple sclerosis as well as peptide sequence identities between EBNA-1 and MBP.

A possible role of EBV infection in the complex pathogenesis of multiple sclerosis remains to be established, but the results in the present study indicate that EBV may participate in the development of the disease.

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HISTORICAL NOTE

A note on the origins of syphilis

Certain early writers suggested that syphilis infected ancient Chinese dynasties, whilst others claim priority for the afflicted populus of the early Romans, alleging that Augustus Caesar was afflicted by hereditary syphilis. But these suppositions are unconfirmed. The sailors with Columbus in 1493 were said to have brought the disease to Spain. It is certain that the Spanish fleet, when they fought for their ruler Alfonso II against the French forces of Charles VIII of France in 1494-5, heavily infected the peoples of Naples. The illness spread rapidly around Europe and mercenaries who in 1496 joined Perkin Warbeck in Scotland and with the support of James IV of Scotland invaded England, bore both arms and the *grandgore* (Old French. grand gorre: grand great + gorre syphilis) as it was called.¹ In 1497 the Minutes of the Town Council of Edinburgh (Phil. Trans. XLII. 421) recognised: "This contagious sickness callit the Grandgor."

The infective and contagious nature of the disease were recognised. The Burgh of Aberdeen issued a ruling that "all licht (loose) women decist fra thar vicis and syne of venerie." A grandgore act was passed in September 1497. There was a suspected endemic of syphilis (treponarid) in 16th century Norway.²

The name syphilis came into common usage. It came from a Latin epic poem *Syphilis, sive Morbus Gallicus*, written by Girolamo Fracastoro or Hieronymus Fracastorius (1483-1553). In his work *De contagione et contagiosis morbis*, he discussed the nature and the spread of infectious diseases, foretelling the germ

theory of disease. A physician, astronomer, and poet of Verona, Fracastoro's poem was written in two volumes, 1525, and in a third published five years later. In 1686 Nahum Tate translated it with the title *Syphilis: or, a Poetical History of the French Disease*. The term syphilis was employed systematically by Fracastoro in his treatise *De Contagione* ii. xi. (1546).

It tells the tale of a brave sailor who navigated a route westward from Spain to "a mighty island in the middle of the sea". The crew shot a flock of colourful birds on the island of Ophir, which belonged to the Sun God. One of the flock escaped and prophetically warned:

"Nor end your sufferings here; a strange Disease,
And most obscene, shall on your Bodies seize . . ."

In Book III, the subject of the poem, the shepherd Syphilus, blasphemes and offends the Sun God. He becomes the first sufferer of the disease.

". . . From whence this Malady its birth receiv'd,
And first th'offending Syphilus was griev'd,
Who rais'd forbidden Altars on the Hill,
And Victims bloud with impious Hands did spill;
He first wore Buboos dreadfull to the sight,
First felt strange Pains and sleepless past the Night;
From him the Malady receiv'd its name."
Book III, 321-32.

continued on page 547