High seroprevalence of Epstein–Barr virus in children with multiple sclerosis

Abstract—We studied seroprevalence and concentrations of Epstein–Barr virus (EBV) antibodies in 147 pediatric patients with multiple sclerosis (MS) and paired controls. The children with MS showed a near-complete seropositivity for EBV antibody against virus capsid antigen (98.6% vs 72.1% in controls, $p = 0.001$) but did not display serologic evidence for a recent EBV infection. EBV antibody concentrations of pediatric patients with MS were significantly higher vs controls.

NEUROLOGY 2006;67:2063–2065

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Epstein–Barr virus (EBV) has been discussed as possible cofactor for the development of multiple sclerosis (MS).[1-9] With regard to MS risk, exposure to environmental factors may be critical in a vulnerable phase before age 15 years.[10] We therefore analyzed the seroprevalence and antibody concentrations of EBV in a cohort of 147 children with MS and matched controls.

Methods. *Patients.* In the context of a longitudinal survey of MS patients with disease onset before age 16 years, blood samples of 147 children with MS were taken at the time of first admission to the neuropediatric department, University of Goettingen, Germany. Diagnosis of MS was established according to the criteria of Poser or McDonald. In the majority of cases, samples were obtained before implementation of immunosuppressive or immunomodulatory therapy. A subgroup of patients was referred from other hospitals and had received different medications before sample acquisition (table 1). Serum probes of children with MS were banked between 1989 and 2004, and samples in a quantity sufficient to perform the complete serologic array of EBV analyses were available from 133 children. In the remaining 14 patients, EBV serologic data were obtained from chart review and comprised at least EBV viral capsid antigen (immunoglobulin G [IgG] anti-VCA) analysis.

Controls. Paired gender-and age-matched (±6 months) control serum probes were obtained from 147 children. Forty-seven of these control samples originated from a serum bank of healthy siblings of patients with adrenoleukodystrophy or neuroblastoma, collected from 1989 to 2005. The remaining 100 control samples were collected from generally healthy, unimpaired children with diverse complaints and negative histories of demyelinating neurologic, immunologic, or severe chronic diseases. These children had been admitted to the pediatric or surgical clinic of the University of Goettingen, Germany, between 1998 and 2005. All children were examined and diagnosed by an experienced pediatrician, who also took each child's history. Complaints are grouped as follows: gastrointestinal (15); headache (12); psychological and psychosomatic (9); traumatic (9); intoxications, including alcohol (8); endocrinologic (7); gynecologic and urologic (6); diverse neurologic (6); syncopes and vertigo (6); diverse acute infections (6); seizures (5); atopic (4); cardiopulmonary (3); orthopedic (2); and obesity (2). Detailed information including age, gender, and individual diagnosis are listed in table E-1 (go to the *Neurology* Web site at www.neurology.org).

The study was approved by the ethics committee of the medical faculty, Georg August University, Goettingen, Germany.

Antibody detection and quantification. Serum samples of patients with MS and controls were stored frozen below −20°C. Patient and matched control samples were analyzed in parallel, blinded to case status, to obtain optimal comparability. Antibody levels were measured by standardized and European Community-certified tests at the departments of virology and medical microbiology at the University of Goettingen, Germany. ELISA classic (Virion Serion, Wuerzburg, Germany) was used to test for EBV-VCA IgG and EBV nuclear antigen 1 (EBNA1) IgG. Amounts of 100 μL of 1:100 diluted sera were analyzed in antigen-coated microwells. Alkaline phosphatase conjugated anti-human IgG from goat (polyclonal) and paranitrophenylphosphate substrate were used. Optical density values were measured with a Tecan Spectra microplate reader for calculation of units per milliliter. Results below cutoff were defined as negative. Indirect immunofluorescence (Meridian, Virotech, Ruesselsheim, Germany) was used to test for EBV early antigen (EA) IgG. Titers >40 were counted positive.

Statistical analysis. Intergroup comparisons for antibody prevalence and chronological classification of serologic findings were performed using logistic regression. To compare antibody concentrations between the two groups, ranked paired *t* tests were used. The Bonferroni–Holm procedure was applied to adjust for multiple testing. Analysis was performed using SAS version 9.1 (SAS Institute Inc, Cary, NC).

Results. *Clinical demographics.* The cohort of children with MS consisted of 98 girls and 49 boys (female to male ratio 2:1), with a mean age at onset of 12.3 years (table 1). The mean age at serum sample acquisition was 13.41 years (range 4.93 to 20.25 years) for the patients with MS and 13.48 years (range 5.40 to 20.36 years) for the matched control patients.

Classification of EBV infection. According to the constellation of antibody reactions toward the different EBV antigens, individuals were classified as EBV negative if antibodies against all three EBV antigens were absent, as remotely infected if antibodies against EBV-VCA and EBNA1 were detectable (irrespective of EBV-EA findings), and as recently infected if antibodies against EBV-VCA and EBV-EA, but not EBNA1, were positive (table 2). Se-

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This work was supported by a grant of the Hertie-Stiftung (GHS 191/00).

Disclosure: The authors report no conflicts of interest.

Received May 19, 2006. Accepted in final form August 8, 2006.

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Table 1 *Characteristics of children with multiple sclerosis (n = 147)*

Characteristics	Number of patients	% of patients
Gender		
Female	98	66.7
Male	49	33.3
Age at first attack, y		
Mean (median)	12.31 (13.13)	
Range	4.04–15.99	
Disease course at time of serum acquisition		
Relapsing remitting	144	97.96
Primary progressive	2	1.36
Secondary progressive	1	0.68
Serum acquisition: time from first attack, y		
Mean (median)	1.10 (0.43)	
Range	0.01–5.87	
CSF findings (available for 132 patients)		
Intrathecal immunoglobulin G fraction >10%	94	71.2
Oligoclonal immunoglobulin G	129	97.7
Immunomodulatory or immunosuppressive medications at time of serum acquisition		
Corticosteroids	14	9.5
Beta-interferons	4	2.7
Azathioprine	2	1.4

Table 2 *Frequency of combinations of EBV serologic results (immunoglobulin G antibodies) and classification (phase of infection) in 147 patients and paired controls*

EBV-VCA	EBNA1	EBV-EA	Classification	Patients	Controls
Negative	Negative	Negative	Negative	2	38
Positive	Negative	Positive	Recent	0	1
Positive	Positive	Positive	Remote*	9	6
Positive	Positive	Negative	Remote†	114	77
Negative	Positive	Negative	NA	0	3
Positive	Negative	Negative	NA	8	22
Positive	Incomplete		NA	14	0

EBV = Epstein–Barr virus; VCA = viral capsid antigen; EBNA1 = EBV nuclear antigen 1; EBV-EA = EBV early antigen; NA = not applicable.

* With signs for reactivation; † without signs for reactivation.

rologic findings not fitting into one of these groups could not be related to a phase of EBV infection.

Classifiable EBV serologic results in patients with MS and paired controls. Fewer children with MS (n = 2, ages 8 and 12 years) than controls (n = 38) were EBV negative ($p = 0.0027$), whereas more patients (n = 123) than controls (n = 83) were remotely infected ($p = 0.0033$). None of the children with MS had been recently infected, whereas one individual from the control cohort had been recently infected.

Antibody prevalences and concentrations. The prevalence and median concentrations of IgG antibodies against EBV-VCA and EBNA1 were significantly higher in the cohort of children with MS than in the control group (table 3). Seropositivity for EBV-EA did not differ significantly between children with MS (9 of 134 analyzed patients, 6.7%) and the matched control group (6 of 134 children, 4.5%).

Discussion. The most striking result of our study is a near-complete seropositivity for EBV-VCA in children with MS compared with only 72% of children in the control group. A seropositivity of 99% to 100% of cases has been reported for adult patients with MS,[5,6] but the fact that healthy adults are EBV seropositive in up to 99% has led to doubts that EBV is causally related to MS. If it is related at all, mainly late infections with EBV occurring during or after puberty have been linked to an increased risk of MS. However, in a recent Canadian study, a high statistical association of EBV and childhood MS was demonstrated.[8] In view of our finding that virtually all children with MS have serologic evidence of a

Table 3 *EBV-IgG antibody prevalences, median concentrations (U/mL) of seropositive individuals, and significance levels (p) in children with multiple sclerosis and paired controls*

	Anti–EBV-VCA			Anti-EBNA1		
	Antibody prevalence, n = 147		Concentration,* n = 94	Antibody prevalence, n = 134		Concentration,* n = 71
	Negative	Positive	Median	Negative	Positive	Median
Patients	2 (1.4%)	145 (98.6%)	62	10 (7.5%)	124 (92.5%)	36
Controls	41 (27.9%)	106 (72.1%)	44	57 (42.5%)	77 (57.5%)	14
p	0.001		0.0002	<0.0001		0.003

Anti–EBV-VCA = Epstein–Barr virus capsid antigen antibody; Anti-EBNA1 = Epstein–Barr virus nuclear antigen 1 antibody.

* Only MS patient/control pairs in which both partners were seropositive were included in the analysis of median concentrations.

prior EBV infection, an EBV infection might be considered a *conditio sine qua non* for the development of MS.

In addition to seroprevalence, our study analyzed EBV antibody concentrations in children with MS. A more prominent immune response to EBV in patients with MS has already been depicted, characterized mainly by elevated concentrations of anti-EBNA1 reported in patients older than 20 years.[2-5,9] We found significantly increased anti-EBNA1 and anti–EBV-VCA IgG concentrations in children with MS. Whether these findings are unique to EBV or are merely representative of an increased propensity for viral reactivation in children with MS needs to be analyzed in further studies.

Because serum was not obtained in close proximity to the first attack in all our patients with MS, we cannot exclude that in some children, EBV infection might have occurred only after disease manifestation. However, the finding that not a single patient in our cohort of children with MS showed a recent EBV infection does not support this hypothesis. Several features of EBV biology make it plausible that EBV could play a role in the pathogenesis of MS. After primary transmission, EBV persistently infects B cells, leading to expression of a broad array of EBV antigens and, subsequently, to proliferation and transformation of EBV-infected B cells. These EBV-infected B cells are highly immunogenic and provoke a vigorous, effective T- and NK-cell response, controlling their proliferation. Given the lifelong persistence of EBV and the possibility of its periodic reactivation, the virus has all features required for a sustained cross-reactive autoimmune response. T-cell responses to EBV infection could include clones that are potentially cross-reactive with self-antigens. Indeed, T-cell cross-recognition between EBV antigens and myelin basic peptides has been demonstrated.[7]

Acknowledgment

The authors thank the children, adolescents, and their parents for their participation.

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