PRELIMINARY COMMUNICATION

Epstein-Barr Virus in Pediatric Multiple Sclerosis

Suad Alotaibi, MD
Julia Kennedy, MSc
Raymond Tellier, MD
Derek Stephens, MSc
Brenda Banwell, MD

Context Infection with common viruses, particularly Epstein-Barr virus (EBV), has been postulated to contribute to the pathobiology of multiple sclerosis (MS). Detailed virological studies in pediatric MS have not been previously reported.

Objective To evaluate whether children with MS are more likely to be seropositive for EBV or other common viruses than their healthy age-matched peers.

Design, Setting, and Patients Case-control study of viral samples collected from March 1994 to February 2003 from 30 pediatric MS patients, 90 emergency department controls matched 3:1 with the MS patients by year of birth, and 53 healthy control children.

Main Outcome Measures Archived serum samples were analyzed for the presence of IgG antibodies directed against EBV viral capsid antigens, nuclear antigens, and early antigens, cytomegalovirus, parvovirus B19, herpes simplex virus, and varicella zoster.

Results Serological evidence for remote EBV infection was present in 83% of pediatric MS patients compared with 42% of emergency department and healthy controls ($P<.001$). Five pediatric MS patients were negative for all 3 EBV antigens. Pediatric MS patients were less likely than controls to have been exposed to herpes simplex virus ($P=.003$), while seropositivity for cytomegalovirus, parvovirus B19, and varicella zoster did not differ between MS patients and controls.

Conclusion These results suggest an association between EBV infection and pediatric MS.

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MULTIPLE SCLEROSIS (MS) IS believed to involve a complex interplay between environmental triggers (such as infections), genetic predisposition, and aberrant immune cell activation. Epidemiological studies suggest that environmental exposure to a putative infectious agent must occur during a specific window of immunological vulnerability in childhood.[1]

Epstein-Barr Virus (EBV) is of particular interest. Acute symptomatic infection with EBV (infectious mononucleosis) can be associated with central nervous system (CNS) demyelination.[2] Although the majority of adult MS patients do not have clinical or serological evidence of acute mononucleosis at the time of MS diagnosis, nearly 100% demonstrate serological evidence of remote EBV infection.[1,3,4] While the association of EBV with adult MS is statistically significant, the pathobiological significance of this observation has been questioned since EBV infects more than 90% of the healthy adult population of Western societies.[5] Infection with EBV occurs in childhood or adolescence in 50% of individuals[6]; the remainder contract EBV during early adulthood. Approximately 5% of all MS patients experience the onset of their disease prior to age 18 years.[7,8] If EBV infection is involved in the initiation of MS, children with MS should demonstrate serological evidence of prior EBV exposure at the time of their MS diagnosis, at an age when the majority of their healthy peers have yet to be exposed to the virus.

METHODS

Participants

Epstein-Barr virus serological studies were available for 30 of 35 children with clinically definite MS (defined by 2 separate and well-documented demyelinating attacks[9]) enrolled in the Pediatric MS Clinic at the Hospital for Sick Children (Toronto, Ontario) as of February 2003. Viral samples were collected from March 1994 to February 2003. Viral serology was not available for 4 children referred from outside Canada and for 1 child in whom initial viral results were inconclusive and archived serum was insufficient for reanalysis.

Selection of control samples was based on the availability of EBV serological results and/or archived serum samples stored in the virology department. To study completely healthy children, we selected samples obtained from bone marrow transplant (BMT) donors. To control for age, we selected samples from an emergency department (ED) cohort matched 3:1 for

Author Affiliations: Department of Pediatrics (Neurology), Al-Sabah Hospital, Shuwaikh, Kuwait (Dr Alotaibi); and Paediatric Laboratory Medicine (Dr Tellier), Department of Paediatrics (Neurology) (Dr Banwell), Brain & Behavior (Ms Kennedy), Population Health Sciences (Mr Stephens), The Hospital for Sick Children, University of Toronto, Toronto, Ontario.
Corresponding Author: Brenda Banwell, MD, Department of Paediatrics (Neurology), Paediatric Multiple Sclerosis Clinic, The Hospital for Sick Children, University of Toronto, 555 University Ave, Toronto, Ontario, Canada, M5G 1X8 (brenda.banwell@sickkids.ca).

age with each MS patient. Selection of control samples was performed by searching, using predetermined search strategies, the Hospital for Sick Children databases for patients entered between 1993 and the end of 2002. All searches were performed blinded to the viral serological results. For the BMT controls the following search criteria were applied: (1) listed as a BMT donor in the database; (2) age between 4 and 18 years; and (3) EBV serology performed. Medical charts were reviewed, and only those BMT donors documented to be completely healthy were then included. For the ED cohort, the following search criteria were used: (1) the patient had been seen in the ED with a presenting diagnosis of rash, pharyngitis, or abdominal pain, and (2) EBV serology was obtained. Medical charts of the potential ED controls were reviewed to ensure that the child was documented to be completely well prior to the acute illness that prompted the ED visit.

Table 1. Characteristics of Patients With Multiple Sclerosis (MS) (n = 30)

Characteristics	No. (%) of Patients
Age at first attack, mean (SD [range]), y	12.04 (3.58 [4.59-17.68])
Age at second attack (MS diagnosis), mean (SD [range]), y	12.71 (3.57 [4.67-18.24])
Season of first attack	
Winter	7 (23)
Spring	8 (27)
Summer	5 (17)
Fall	10 (33)
First attack signs and symptoms	
Isolated optic neuritis	6 (21)
Isolated transverse myelitis	1 (3)
Monosymptomatic (other than isolated optic neuritis and transverse myelitis)	7 (23)
Polysymptomatic	13 (43)
Acute disseminated encephalomyelitis	3 (10)
Timing of viral sample acquisition	
Within 6 mo of first attack	8 (27)
0-6 mo after second attack	13 (43)
6-12 mo after second attack	1 (3)
1-2 y after second attack	3 (10)
>2 y after second attack	5 (17)
Time from first attack, mean (SD [range]), y	1.36 (1.74 [0.01-5.69])
Country of birth	
Canada	22 (73)
Other*	8 (27)
Cerebrospinal fluid oligoclonal bands	
Negative	2
Positive	4
Not available†	24
Family history of MS	5 (17)
First degree	1
Second degree	2
Third/fourth degree	2
No family history of MS‡	25 (83)
Medications at time of viral sample acquisition§	
Therapy for unrelated conditions (erythromycin, nystatin, medroxyprogesterone)	3 (10)
≥1 Doses of corticosteroids	6 (20)
No medications	21 (70)

*Seven patients immigrated to Canada during childhood, and 1 child is a resident of Greece. The percentage of Epstein-Barr virus–positive, non-Canadian-born MS patients is 81%, which does not differ from the MS cohort as a whole (83% Epstein-Barr virus positive).
†Cerebrospinal fluid acquisition in children presenting with acute neurological deficits is typically performed by the primary care pediatrician to exclude infection, and often prior to confirmation of demyelination by magnetic resonance imaging. As a result, cerebrospinal fluid oligoclonal band studies are often not available.
‡Given the young age of the parents and first-degree relatives of pediatric MS patients, it is possible that some relatives will be diagnosed with MS in the future.
§None of the children had received treatment with MS-targeted, disease-modifying therapies at the time of viral sample acquisition (interferons or glatiramer acetate).

Detection of Antiviral Antibodies

Serum samples from all participants were analyzed in the licensed clinical microbiology laboratory at the Hospital for Sick Children in batches, blinded to case status. Samples were analyzed using standardized enzyme-linked immunosorbent assay (ELISA) kits for IgG antibodies directed against EBV capsid (EBV-VCA), nuclear (EBV-EBNA), and early antigens (EBV-EA) (DiaSorin, Stillwater, Minn). Archived samples from the MS cohort and ED controls, obtained and stored at the time of initial EBV sampling, were then retrieved and analyzed for the presence of IgG antibodies directed against cytomegalovirus (CMV) (Zeus Scientific, Raritan, NJ), parvovirus B19 (Biotrin International Ltd, Mount Merrion, Co. Dublin, Ireland), varicella zoster virus (VZV) (Zeus Scientific), and herpes simplex virus (HSV) (BioChem ImmunoSystems Italia SPA, Casalecchio di Reno, Italy). Twenty of the control samples originally analyzed for EBV using immunofluorescence assays were reanalyzed by ELISA to ensure uniform methodology. The HSV ELISA kit does not discriminate infection with HSV-I from HSV-2. One MS patient had insufficient serum to analyze for VZV, another insufficient serum for HSV, and a third patient had no archived serum available. The remaining 27 MS patients and 67 of the ED controls had sufficient archived serum for analysis of the entire viral panel.

Patients were classified as "remotely infected" if EBV antibodies against both VCA and EBNA (irrespective of EA) were detected, "recently infected" if antibodies against VCA and EA (but not EBNA) were detected, and "EBV-naive" if antibodies against all 3 EBV antigens were absent. Samples were viewed as uninterpretable if results did not conform to

1 of the 3 possibilities. Serological test results for CMV, parvovirus B19, VZV, and HSV were recorded as positive or negative based on interpretive criteria provided by the manufacturer.

Statistical Analysis

Logistic regression analysis was performed comparing remote EBV infection between MS patients and BMT donors and comparing the number of MS patients and controls with negative serological results for EBV. Conditional logistic regression analysis for a matched case-control design was performed comparing the MS patients with the age-matched ED controls for EBV, CMV, parvovirus B19, VZV, and HSV. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Statistical analysis was performed using SAS version 8.2 (SAS Institute Inc, Cary, NC).

The study was approved by the Research Ethics Board of The Hospital for Sick Children. Individual consent for analysis of archived specimens was not required.

RESULTS

Subjects

The mean time from the first MS attack to viral sample acquisition was 1.36 years (TABLE 1). All MS children experienced multiple attacks, and although many are now receiving MS-targeted therapies, none were receiving these treatments at the time of sample acquisition. None of the MS patients reported a history of symptoms compatible with acute mononucleosis. The mean age of the MS and matched ED cohorts was similar as expected (13.40 and 13.37 years, respectively), but the BMT patients were younger (10.30 years) (TABLE 2). There were more girls in the MS and ED cohorts but more boys in the BMT control group.

Table 2. Demographic Features of Patients With Multiple Sclerosis (MS) and Control Groups

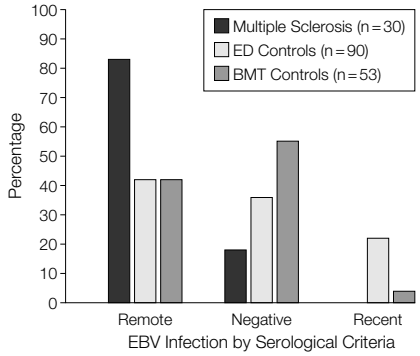
Demographic Features	Patients With MS (n = 30)	Emergency Department Controls (n = 90)	Bone Marrow Transplant Controls (n = 53)
Age, mean (SD), y*	13.40 (3.63)	13.37 (3.62)	10.30 (3.78)
Female-male ratio	1.31:1	1.57:1	0.66:1
Residence, No. (%)			
Toronto	17 (57)	81 (90)	20 (38)
Ontario (not Toronto)	11 (37)	6 (6)	28 (52)
Other	2 (6)†	3 (4)‡	5 (10)§

*Age refers to the age of the patient at the time the virology sample was obtained. The age range for all 3 groups was 4 to 18 years.
†One patient lives in Greece, and 1 patient lives in eastern Canada.
‡One patient lives in the United States, 1 patient lives in England, and 1 patient lives in eastern Canada.
§Five patients live in eastern Canada.

Antibodies Against EBV

As shown in FIGURE 1, remote EBV infection was identified in 83% of the MS cohort compared with 42% of the BMT controls (OR, 7.04; 95% CI, 2.3-21.3; $P<.001$) and 42% of the healthy, age-matched ED controls (OR, 8.7; 95% CI, 2.5-30.3; $P<.001$). Only 17% of the MS patients were seronegative for EBV, compared with 55% of the BMT donor cohort (OR, 0.17; 95% CI, 0.06-0.5; $P<.001$) and 36% of the ED controls (OR, 0.27; 95% CI, 0.075-0.987; $P=.04$). As expected, recent infection was highest in the ED cohort (22%) in whom serological testing was performed due to clinically suspected acute EBV infection. Recent infection with EBV was not found in children with MS, even those sampled at the time of their first demyelinating episode.

Figure 1. Comparison of Epstein-Barr Virus Serological Results Between Pediatric Multiple Sclerosis Patients and Controls



Epstein-Barr virus (EBV) serological results in children with clinically definite multiple sclerosis, emergency department (ED) controls, and bone marrow transplant (BMT) controls. Patients were classified as "remotely infected" if EBV antibodies against both capsid (VCA) and nuclear (EBNA) antigens (irrespective of early antigens [EA]) were detected; "recently infected" if antibodies against VCA and EA (but not EBNA) were detected; and "EBV-negative" if antibodies against all 3 EBV antigens were absent. Children with multiple sclerosis were more likely to be positive for remote EBV infection than ED ($P<.001$) or BMT controls ($P<.001$) and less likely to be EBV-negative than ED ($P=.04$) or BMT controls ($P<.001$).

Antibodies Against HSV, Parvovirus B19, VZV, and CMV

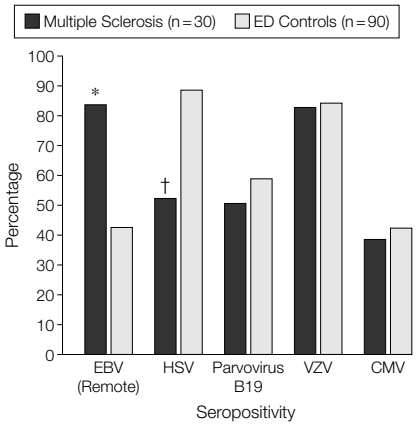
As shown in FIGURE 2, MS patients did not differ from controls for the prevalence of antibodies against parvovirus B19, VZV, or CMV, but were less likely to have been exposed to HSV than the control cohort (52% vs 88%) (OR, 0.14; 95% CI, 0.04-0.51; $P=.003$).

COMMENT

Pediatric MS patients are significantly more likely to have experienced EBV infection than their peers. Our results may be interpreted in several ways, including the following: (1) infection with EBV initiates or propagates MS pathogenesis; (2) MS leads to an increased susceptibility to B-cell infection with EBV; or (3) a common mechanism exists leading to heightened susceptibilities to early EBV infection and early-onset MS.

The pathogenesis of MS may relate to immune responses to environmental agents such as viruses encountered during the pediatric-age window of risk.[5,10-14] There are several features of EBV that make it biologically plausible that it could play a role in MS. Exposure to EBV results in persistent B-cell infection, expansion of EBV-transformed B-cell clones, and the production of antibodies directed against specific EBV viral antigens, as well as lifelong T-cell surveillance of infected B cells.[15] The presence of EBV antigen–responsive T cells is not inher-

Figure 2. Comparison of Seropositivity for Common Childhood Viruses Between Children With Multiple Sclerosis and Healthy Age-Matched Controls



Epstein-Barr virus (EBV) (remote infection), herpes simplex virus (HSV), parvovirus B19, varicella zoster (VZV), and cytomegalovirus (CMV) serological results in multiple sclerosis patients and emergency department (ED) controls. Children with multiple sclerosis were more likely to be positive for remote EBV infection than ED controls (*$P<.001$) and less likely to be HSV-positive (†$P=.003$). There was no difference in seropositivity for parvovirus, VZV, or CMV between the multiple sclerosis and ED cohorts.

ently pathogenic, as they are present in a quiescent state in most healthy EBV-positive adults. The pathogenic potential of these T cells requires activation, possibly through molecular mimicry. A pentapeptide sequence found in the EBV nuclear antigen shares sequence homology with an epitope of myelin basic protein, a major component of the myelin sheath.[15] Furthermore, EBV induces B-cell surface expression of alpha-B crystallin, a protein recently identified as a major autoantigen constituent that is abnormally expressed in brains of patients with MS.[16] It is thus conceivable that exposure to EBV may lead to a misdirected host immune response against self-antigens in the CNS, such as alpha-B crystallin and/or myelin basic protein.

Epstein-Barr virus is not the only virus implicated in MS and clearly is not a requisite trigger, as evidenced by the 5 EBV-negative pediatric MS patients. A role for human herpesvirus 6 (HHV-6) has been suggested by studies of HHV-6 expression in CNS tissue and by the identification of increased HHV-6 antibody titers in serum samples of MS patients.[17] Human herpesvirus 6 variant A infection leads to activation of the EBV genome in EBV-positive B cells,[18] raising the possibility that multiple viral exposures may act in concert. However, the literature on HHV-6 is complicated by differences in methodology between studies,[19] and by the fact that nearly 100% of the population is infected with HHV-6 by the age of 2 years. Of greater interest would be the study of HHV-6 replicative/latency status, which would require molecular methods such as polymerase chain reaction techniques.[20] Such analyses are planned. Although many viral agents other than EBV, including *Chlamydia pneumoniae,* have been studied in MS, strong associations have yet to be documented, owing in part to differences in methodology and patient populations.[21]

It is possible that the association between EBV infection and MS relates to increased exposure or susceptibility to EBV infection in MS-affected children, rather than a causal role for EBV in MS pathogenesis. However, pediatric MS patients do not seem to have an increased susceptibility or exposure to viruses in general, as evidenced by the similarity in seropositivity rates between MS patients and controls for parvovirus B19, CMV, and VZV.

Another important issue is whether EBV infection initiates, rather than propagates, the immunological processes involved in MS. A study of EBV infection in 3 million US military personnel demonstrated a strong positive association between EBV antibodies and MS risk in samples collected more than 5 years before MS diagnosis.[22] Further support for the role of EBV in the initiation of MS pathogenesis will be sought by studying EBV serology in children presenting with initial acute CNS demyelination: children subsequently diagnosed with MS would be expected to show evidence of remote EBV infection even at the time of their first attack.

An interesting observation in our study was the fact that healthy children were more likely than pediatric MS patients to be seropositive for HSV. Although our methodology does not allow discrimination between HSV-1 and HSV-2, most participants in our study were younger than 16 years and few were likely to be sexually active. Thus, their positive serological test results most probably reflect previous infection with HSV-1. It has been hypothesized that HSV-1 immunity is protective against MS.[23] If the sequence of viral exposures in childhood is important in MS pathobiology, then perhaps pediatric MS patients experience EBV infection without the "protective" benefit of early HSV-1 infection.

Although demographic data on our control cohorts are limited, it is unlikely that demographic differences between controls and MS patients would account for the marked difference in EBV seropositivity. In fact, the demographic features of our 2 control cohorts were not matched to each other, yet EBV seroprevalence was identical. Furthermore, the seroprevalence of EBV in our controls was similar to that of other pediatric control cohorts in North American studies of EBV.[6,24] In addition, although the sample size of the current study was small, significant results were obtained. Validation of these results requires a larger, prospective study of multiple viruses in healthy children and pediatric MS patients.

Author Contributions: As principal investigator, Dr Banwell had full access to all data in the study and takes responsibility for the integrity and accuracy of the data and analyses.
Study concept and design: Banwell.
Acquisition of data: Alotaibi, Kennedy, Tellier, Banwell.
Analysis and interpretation of data: Kennedy, Tellier, Stephens, Banwell.
Drafting of the manuscript: Alotaibi, Kennedy, Banwell.
Critical revision of the manuscript for important intellectual content: Alotaibi, Kennedy, Tellier, Stephens, Banwell.
Statistical expertise: Stephens.
Obtained funding: Banwell.
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Supervision: Tellier, Banwell.
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> Truth is a torch, but a terrific one; therefore we all try to grasp it with closed eyes, fearing to be blinded.
> —Johann Wolfgang Von Goethe (1749-1832)