Epstein-Barr Virus in Pediatric Multiple Sclerosis

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

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 individuals6; 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 attacks8) 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

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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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.

**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 sample 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

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

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

*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 (53% 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).
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 cohorts was similar as expected (13.40 mean age of the MS and matched ED controls) 20% 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.

**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.1-3,10-14 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-

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

<table>
<thead>
<tr>
<th>Demographic Features</th>
<th>Patients With MS (n = 30)</th>
<th>Emergency Department Controls (n = 90)</th>
<th>Bone Marrow Transplant Controls (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y*</td>
<td>13.40 (3.63)</td>
<td>13.37 (3.62)</td>
<td>10.30 (3.78)</td>
</tr>
<tr>
<td>Female-male ratio</td>
<td>1.31:1</td>
<td>1.57:1</td>
<td>0.66:1</td>
</tr>
<tr>
<td>Residence, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toronto</td>
<td>17 (57)</td>
<td>81 (90)</td>
<td>20 (38)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (6)†</td>
<td>3 (4)‡</td>
<td>5 (10)§</td>
</tr>
</tbody>
</table>

*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.

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**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).

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identification of increased HHV-6 antibo
dy titers in serum samples of MS pa
tients. Human herpesvirus 6 variant A
infection leads to activation of the EBV
genome in EBV-positive B cells, rais
ing the possibility that multiple viral ex
posures may act in concert. However, the
literature on HHV-6 is complicated by dif
ferences in methodology between studi
es, 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 poly
merase chain reaction techniques. Such
analyses are planned. Although many vi
ral agents other than EBV, including
Chlamydia pneumoniae, have been studi
ed in MS, strong associations have yet
to be documented, owing in part to dif
ferences in methodology and patient
populations.

It is possible that the association be
 tween EBV infection and MS relates to
increased exposure or susceptibility to
EBV infection in MS-affected chil
dren, rather than a causal role for EBV
in MS pathogenesis. However, pediat
ric MS patients do not seem to have an
increased susceptibility or exposure to
viruses in general, as evidenced by the
similarity in seropositivity rates be
tween MS patients and controls for par
ovirus, VZV, or CMV between the multiple scle
rosis and ED cohorts.

Epstein-Barr virus (EBV) (remote infection), herpes sim
plex virus (HSV), parvovirus B19, varicella zoster (VZV),
and cytomegalovirus (CMV) serological results in mul
tiple 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 < 0.001) and less likely to be HSV-positive (P = 0.003). There was no difference in seropositivity for parvovirus, VZV, or CMV between the multiple sclerosis and ED cohorts.

Epstein-Barr virus (EBV) is not the only vi
rus 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

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, Teller, Banwell.

Analysis and interpretation of data: Kennedy, Teller, Stephens, Banwell.

Drafting of the manuscript: Alotaibi, Kennedy, Banwell.

Critical revision of the manuscript for important intellectual content: Alotaibi, Kennedy, Teller, Stephens, Banwell.

Statistical expertise: Stephens.

Obtained funding: Banwell.

Administrative, technical, or material support: Alotaibi, Kennedy.

Supervision: Teller, Banwell.

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REFERENCES

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)