Multiple Sclerosis and Epstein-Barr Virus

Lynn I. Levin, PhD, MPH
Kassandra L. Munger, MSc
Mark V. Rubertone, MD, MPH
Charles A. Peck, MD
Evelyne T. Lennette, PhD
Donna Spiegelman, DSc
Alberto Ascherio, MD, DrPH

Context Infection with Epstein-Barr virus (EBV) has been associated with an increased risk of multiple sclerosis (MS), but the temporal relationship remains unclear.

Objective To determine whether antibodies to EBV are elevated before the onset of MS.

Design, Setting, and Population Nested case-control study conducted among more than 3 million US military personnel with blood samples collected between 1988 and 2000 and stored in the Department of Defense Serum Repository. Cases were identified as individuals granted temporary or permanent disability because of MS. For each case (n=83), 2 controls matched by age, sex, race/ethnicity, and dates of blood sample collection were selected.

Main Outcome Measures Antibodies including IgA against EBV viral capsid antigen (VCA) and IgG against VCA, nuclear antigens (EBNA complex, EBNA-1, and EBNA-2), diffuse and restricted early antigens, and cytomegalovirus.

Results The average time between blood collection and MS onset was 4 years. The strongest predictors of MS were serum levels of IgG antibodies to VCA or EBNA complex. The risk of MS increased monotonically with these antibody titers; relative risk (RR) in persons in the highest category of VCA (≥2560) compared with those in the lowest (≤160) was 19.7 (95% confidence interval [CI], 2.2-174; *P* for trend=.004). For EBNA complex titers, the RR for those in the highest category (≥1280) was 33.9 (95% CI, 4.1-283; *P* for trend <.001) vs those in the lowest category (≤40). Similarly strong positive associations between EBV antibodies and risk of MS were already present in samples collected 5 or more years before MS onset. No association was found between cytomegalovirus antibodies and MS.

Conclusion These results suggest a relationship between EBV infection and development of MS.

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ELEVATIONS IN ANTI–EPSTEIN Barr virus (EBV) serum antibody titers occurring several years before diagnosis have been characteristically found in diseases probably caused by EBV, such as Burkitt lymphoma[1] and nasopharyngeal carcinoma,[2] and in Hodgkin disease.[3] Anti-EBV antibodies are elevated in individuals with multiple sclerosis (MS),[4,5] and we recently reported a premorbid increase in a small study,[6] but it remains uncertain whether these elevations predate disease onset. We therefore conducted a larger prospective investigation using serial blood samples collected several years before onset of MS.

METHODS

Study Population

The source population for the present study is more than 3 million US military personnel whose blood samples are stored at −30°C in the Department of Defense Serum Repository.[7] This repository contains more than 30 million serum specimens from active-duty and reserve personnel of the US military collected at entry and, on average, every 2 years thereafter since 1988. The research protocol was approved by the relevant institutional review board, which waived the need for consent to use blood products in a study.

Case Ascertainment and Selection of Controls

Cases were identified by searching the computerized database of the US Army Physical Disability Agency for active-duty personnel granted temporary or permanent disability because of MS and by reviewing medical records. We classified cases as definite MS if there was a history of 2 or more attacks, a diagnosis of MS made by a neurologist, and a positive magnetic resonance imaging (MRI) result or if the final diagnosis in the record was specified as definite MS, clinical definite MS, or laboratory-supported definite MS.[8] Cases were classified as probable MS if they did not meet the criteria for definite MS but had at least 2 of the following: history of 2 or more attacks, positive MRI result, and diagnosis of MS made by a neurologist. These criteria (definite or probable MS) were met by 118 cases, 83 of whom had at least 1 serum sample collected before onset of MS symptoms (defined as the earliest neurological symptom ever reported) and were included in the study. For each of these 83 cases, we

Author Affiliations: Division of Preventive Medicine, Walter Reed Army Institute of Research (Dr Levin), Army Medical Surveillance Activity, US Army Center for Health Promotion and Preventive Medicine (Dr Rubertone), and US Army Physical Disability Agency (Dr Peck), Washington, DC; Departments of Nutrition (Ms Munger and Dr Ascherio) and Epidemiology (Drs Spiegelman and Ascherio), Harvard School of Public Health, Boston, Mass; and Virolab Inc, Berkeley, Calif (Dr Lennette).
Corresponding Author and Reprints: Alberto Ascherio, MD, DrPH, Harvard School of Public Health, Nutrition Department, 665 Huntington Ave, Boston, MA 02115 (e-mail: Alberto.Ascherio@channing.harvard.edu).

identified the earliest available serum sample (baseline sample) plus up to 2 additional samples collected before onset of MS and the first sample collected after onset of MS. For each of the 83 cases, we randomly selected 2 controls matched by age (±1 year), sex, race/ethnicity (white, black, Hispanic, or other), and dates of blood collection (±30 days). For serial samples, each blood sampling date was matched to within 30 days.

Laboratory Analyses

Serum samples from MS cases and controls were sent to the laboratory in triplets containing the case and the 2 matched controls in random order without identification of case-control status. Immunoglobulin G and IgA antibodies to EBV viral capsid antigen (VCA) and anti–early antigen complex (diffuse [EA-D] and restricted [EA-R]) were determined by indirect immunofluorescence[9,10]; IgG antibodies against the EBV nuclear antigen (EBNA) complex and 2 of its individual members, EBNA-1 and EBNA-2, were determined by anticomplement immunofluorescence.[11] Immunoglobulin G antibody titers against cytomegalovirus (CMV) were also determined to assess the specificity of any association that may be found between MS and EBV serology.[12]

Statistical Analyses

Geometric mean antibody titers (reciprocal of the dilution) were compared between cases and controls using generalized linear models.[13] We used conditional logistic regression to estimate the relative risk (RR) of MS associated with serum levels of specific antibody titers. The main regression analyses were conducted using the antibody titers as categorical variables; further analyses were conducted using the base 2 logarithm of the reciprocal of the dilution as a continuous variable (on this scale, a 1-unit increase corresponds to a 2-fold change in titers). Antibody titers were categorized by the highest dilution that tested positive, and each doubling (eg, 20, 40, 80) formed a category. The lowest and highest categories were collapsed because of small numbers or lack of cases in these categories. The differences in antibody titers between samples collected before or after MS onset were tested by paired *t* test. All *P* values are 2-tailed and significant at $P<.05$. We used SAS version 6.12 (SAS Institute Inc, Cary, NC) for all analyses.

Table 1. Characteristics of Multiple Sclerosis Cases and Controls

	Cases (n = 83)	Controls (n = 166)
Sex, No. (%)		
Male	54 (65)	108 (65)
Female	29 (35)	58 (35)
Age at collection of baseline blood, y		
Mean (SD)	24 (5.1)	24 (5.1)
Range	17-39	17-39
Race/ethnicity, No. (%)		
White	50 (60)	100 (60)
Black	29 (35)	58 (35)
Hispanic	1 (1)	2 (1)
Other	3 (4)	6 (4)
Education, No. (%)		
High school	65 (78)	127 (77)
Some college	7 (8)	11 (7)
Completed college	7 (8)	22 (13)
Graduate school	4 (5)	6 (4)

RESULTS

Baseline characteristics of cases and controls are shown in TABLE 1. All cases and 96% of the controls had evidence of EBV infection (VCA IgG ≥20) at baseline ($P=.08$). For cases, the mean (SD) age at MS onset was 27 (5.5) years (range, 18-41 years). Certainty of diagnosis was definite in 53 (64%) and probable in 30 (36%). Mean (SD) time between baseline blood collection and MS onset was 4.0 (2.4) years (range, <1-11 years). The baseline geometric mean serum antibody titers to EBV were consistently higher among individu-

Table 2. Geometric Mean Titers of Antibodies in Baseline Serum Samples

	All Subjects			Cases With Blood Collected ≥5 y Before MS Onset		
Antibodies	Cases (n = 83)	Matched Controls (n = 165)*	*P* Value	Cases (n = 28)	Matched Controls (n = 56)	*P* Value
IgG to EBV VCA	909	603	<.001	840	552	.02
IgA to EBV VCA	3.0	2.8	.26	3.2	2.8	.31
EBNA complex	537	224	<.001	565	197	<.001
EBNA-1	385	179	<.001	431	147	<.001
EBNA-2	26	17	.02	27	19	.38
Diffuse early antigen	4.3	3.5	.17	4.9	4.0	.20
Restricted early antigen	4.1	3.2	.06	5.4	3.2	.02
Cytomegalovirus	16	15	.56	14	16	.65

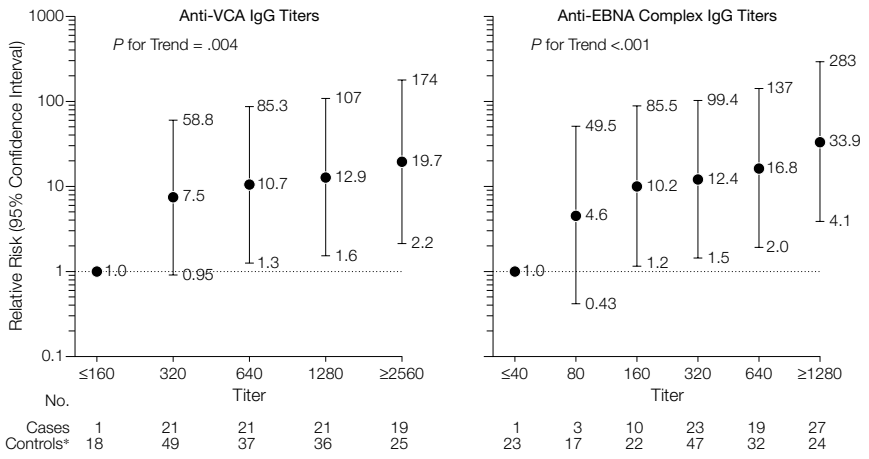
Abbreviations: EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; MS, multiple sclerosis; VCA, viral capsid antigen.
*Baseline antibody titers were missing for 1 control.

als who later developed MS than among their matched controls, whereas there was no difference in antibodies to CMV (TABLE 2). Similar results were observed in analyses restricted to cases with blood samples collected at least 5 years before the onset of MS (Table 2). The risk of MS increased monotonically with increasing serum levels of antibodies to VCA and EBNA complex. Compared with individuals with the lowest antibody titers, the RR was 19.7 (95% confidence interval [CI], 2.2-174; *P* for trend=.004) for individuals in the highest category of VCA IgG and was 33.9 (95% CI, 4.1-283; *P* for trend <.001) for individuals in the highest category of EBNA complex (FIGURE). A strong positive association was also found with EBNA-1 (RRs for titers from ≤40 to ≥1280: reference, 6.1, 8.1, 6.3, 11.9, and 16.7), whereas weaker associations were found for VCA IgA (RRs for titers ≤2.5, 10-20, and ≥40: reference, 0.0, and 3.6), EBNA-2 (RRs for titers from ≤2.5 to ≥160: reference, 1.4, 1.7, 3.1, 1.7, 3.1, and 2.5), EA-D (RRs for titers ≤2.5, 5-20, 40-80, and ≥160: reference, 0.7, 1.5, and 2.4), and EA-R (RRs for titers ≤2.5, 5-20, 40-80, and ≥160: reference, 2.3, 1.5, and 10.2). There was no association between antibodies to CMV and risk of MS (RRs for titers ≤5, 10-20, 40-80, and ≥160: reference, 2.3, 0.91, and 0.95).

In analyses using the log-transformed antibody titers as continuous variables, strong associations were found with IgG antibodies to VCA, EBNA complex, and EBNA-1 (TABLE 3); these associations did not vary significantly by sex, age, or race/ethnicity. When the serum titers of different anti-EBV antibodies were included simultaneously in a regression model, only the EBNA complex and EBNA-1 titers retained statistical significance (these titers were not included at the same time because EBNA immunofluorescence is mostly accounted for by EBNA-1 antibodies[11] so that these titers are highly correlated). The EBNA-1/EBNA-2 ratio was not significantly associated with risk of MS. Results of analyses restricted to cases of definite MS were not materially different.

Finally, we used the repeated serological determinations to examine whether specific antibody titers varied among cases and whether there was evidence of EBV reactivation before onset of MS symptoms. For all antibodies, titers were similar in the earliest available sample (mean, 3.6 years before MS onset) and the sample collected after MS onset (mean, 1.0 years after MS onset). The geometric mean titers in the pre-onset and post-onset samples, respectively, were as follows: for VCA IgG, 905 and 896 (*P*=.88 by paired *t* test); for VCA IgA, 3 and 3 (*P*=.32); for EBNA, 561 and 561 (*P*>.99); for EBNA-1, 400 and 405 (*P*=.92); for EBNA-2, 26 and 27 (*P*=.72); for EA-D, 4 and 4 (*P*=.76); for EA-R, 4 and 3 (*P*=.33); and for CMV, 18 and 17 (*P*=.68). Positive IgA titers to VCA and IgG titers to EA-D reflect repeated infection or reactivation. Overall, the proportion of cases and controls with at least 1 positive titer (≥5) before MS onset (index date in controls) was not significantly different in cases (6% for VCA IgA and 31% for EA-D) and controls (4% and 21%,

Figure. Relative Risk of Multiple Sclerosis According to Anti-VCA IgG and Anti-EBNA Complex IgG Antibody Titers in Baseline Serum Samples



VCA indicates viral capsid antigen; IgG, immunoglobulin G; and EBNA, Epstein-Barr nuclear antigen. Asterisk indicates that baseline antibody titers were missing for 1 control.

Table 3. Relative Risk of MS Corresponding to a 4-Fold Increase in Anti-EBV and Anti-CMV Baseline Serum Antibody Titers

	All Cases (n = 83)*		Cases With Blood Collected ≥5 y Before MS Onset (n = 28)†	
Antibodies	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
IgG to EBV VCA	1.8 (1.2-2.8)	<.001	1.9 (0.91-4.0)	.06
IgA to EBV VCA	1.4 (0.74-2.5)	.32	1.6 (0.62-4.1)	.33
EBNA complex	2.3 (1.6-3.4)	<.001	2.6 (1.3-5.0)	<.001
EBNA-1	1.7 (1.3-2.3)	<.001	2.2 (1.2-4.1)	.002
EBNA-2	1.4 (1.1-1.9)	.02	1.3 (0.82-2.0)	.27
Diffuse early antigen	1.2 (0.89-1.7)	.22	1.2 (0.72-2.1)	.47
Restricted early antigen	1.5 (1.0-2.3)	.05	3.1 (1.1-8.8)	.008
Cytomegalovirus	1.1 (0.83-1.4)	.57	0.91 (0.53-1.6)	.72

Abbreviations: CI, confidence interval; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; MS, multiple sclerosis; RR, relative risk; VCA, viral capsid antigen.
*Conditional logistic regression: EBV and CMV titers were missing for 1 control (n = 165).
†Conditional logistic regression: n = 56 controls.

respectively). However, cases were more likely than controls to have at least 1 sample with a titer of at least 80 (for VCA IgA, RR, 8.0; 95% CI, 1.3-50.4; $P=.03$; for EA-D, RR, 4.2; 95% CI, 1.6-11.4; $P=.005$).

COMMENT

These results confirm those obtained in a smaller study of women with MS.[6] Although the date of onset of MS is difficult to establish accurately, and many patients at the time of clinical onset have multiple silent MRI lesions,[14] the lack of variation in our study in anti-EBV antibody titers between samples collected 6 to 11 years before the estimated MS onset and later samples suggests that the increased antibody response to EBV occurs early in relation to the pathological process that leads to demyelination and clinical disease. Furthermore, all cases were already seropositive at the time of collection of the first blood sample (mean, 4 years before MS onset) and appeared to have stable antibody titers, suggesting that there is a long lag time between infection with EBV and occurrence of MS.

The pattern of antibody response that we observed among individuals who developed MS is different from the pattern observed in immunocompromised hosts or in chronic infectious mononucleosis, where there are elevated anti–EBNA-2 and reduced anti–EBNA-1 titers,[11] and from that observed in Burkitt lymphoma, where there are prediagnostic elevations of anti-VCA but not anti-EBNA antibodies.[1] Rather, the simultaneous elevation of titers to VCA and EBNA-1 suggests a more severe or more recent primary infection or reactivation of infection accompanied by a vigorous cellular immune response.[15-17] A similar pattern of anti-VCA and anti-EBNA IgG elevation in prediagnostic sera has been associated with risk of Hodgkin disease[3] and nasopharyngeal carcinoma,[18] but in the latter the strongest predictors of risk are IgA antibodies to VCA.[19] As previously discussed, these results support a role for EBV in the etiology of MS.[6]

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Acquisition of data: Munger, Rubertone, Peck, Lennette, Spiegelman, Ascherio.
Analysis and interpretation of data: Levin, Munger, Peck, Spiegelman, Ascherio.
Drafting of the manuscript: Levin, Rubertone, Ascherio.
Critical revision of the manuscript for important intellectual content: Levin, Munger, Peck, Lennette, Spiegelman, Ascherio.
Statistical expertise: Levin, Spiegelman, Ascherio.
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Study supervision: Peck, Spiegelman, Ascherio.
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