Published in final edited form as:

Mult Scler. 2012 August ; 18(8): 1185–1187. doi:10.1177/1352458511433920.

Epstein-Barr virus neutralizing antibody levels and risk of multiple sclerosis

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Abstract

Previous infection with Epstein-Barr virus (EBV) and infectious mononucleosis are established multiple sclerosis (MS) risk factors, and elevated serum titers of anti-EBV nuclear antigen antibodies in healthy adults are strongly correlated with future MS risk. In this prospective study, we investigated the association between EBV neutralizing antibodies and MS risk. MS risk tended to be higher in individuals with high titers of neutralizing antibodies compared to those with low titers (RR=2.2, 95% CI:0.97, 5.1). This association was attenuated after adjustment for anti-EBNA1 IgG Ab titers (RR=1.4, 95% CI: 0.5, 3.5). This preliminary finding warrants further study in a larger population.

Keywords

multiple sclerosis; Epstein-Barr virus; neutralizing antibodies; Epstein-Barr nuclear antigen; glycoprotein 350; infectious mononucleosis

Introduction

The association between Epstein-Barr virus (EBV) infection and increased multiple sclerosis (MS) risk is well established.^{1, 2} Further, individuals with a history of infectious mononucleosis (IM), a manifestation of EBV infection in adolescence/adulthood, have a 2-fold increased MS risk³. An experimental vaccine comprising the EBV glycoprotein 350 (gp350)⁴, a viral antigen expressed on the EBV envelope that facilitates EBV entry into B cells by binding CD21/CR2⁵, has recently been tested in a double blind trial of 181 EBV negative healthy young adults followed for 18 months for evidence of EBV infection and IM. Although almost all individuals seroconverted during the follow-up, the vaccine had a 78% efficacy for IM prevention.⁶ The efficacy of this vaccine in preventing IM suggests that among non-vaccinated individuals the ability to mount a strong immune response to gp350 could reduce the risk of IM and MS.

In a previous study, no difference in serum titers of EBV neutralizing antibodies was found in MS cases as compared to controls⁷, but there have been no reports to date investigating

whether the EBV neutralizing activity in apparently healthy individuals predicts their MS risk. To better understand the relationship between EBV infection, the host immune response and MS, we measured gp350 neutralizing antibody capabilities in a nested case-control study of MS patients and matched controls with serum collected before MS diagnosis.

Materials and Methods

Study population

The source population includes the 32,826 Nurses' Health Study (NHS) cohort participants and the 28,613 Nurses' Health Study II (NHS II) participants who provided blood samples.⁸ A total of 149 incident cases of definite and probable MS were documented between baseline and December 1999 among these women as previously described.⁸ For each case, we randomly selected two women without MS, matched by year of birth and study cohort. We restricted our analyses to the 31 women with a blood sample collected prior to MS diagnosis and their matched controls; in 18 of these women blood was collected before the onset of the first neurological symptoms attributed to MS.

EBV neutralizing antibodies

EBV neutralizing antibodies were measured using a competition ELISA as previously described⁹. Briefly, the assay measures the ability of the EBV neutralizing monoclonal antibody, mAb 72A1, directed at EBV gp350, to compete with neutralizing antibodies in patients' sera for binding to recombinant gp350. As such, neutralizing activity is reported in ng/ml of 72A1 equivalents. Higher levels of 72A1 binding suggest lower binding of gp350 by serum antibodies, thus suggesting lower neutralizing antibody levels and lower neutralizing activity. Overall, the coefficient of variation was 15% with batch-specific estimates ranging from 5–19%.

Gp350 antibodies and anti-EBNA antibodies

Gp350 antibody titers were measured by ELISA as previously described.⁹ IgG antibody titers to anti-EBNA were measured as part of a previous investigation of anti-EBV Ab titers (anti-EBNA1, anti-EBNA2, anti-EA-D, anti-EA-R and anti-VCA) and MS risk.⁸

Statistical analysis

The relationship between gp350 antibodies or EBV neutralizing antibody inhibition and MS risk was assessed via conditional logistic regression to obtain relative risks (RRs) and 95% confidence intervals (95% CIs). Antibody titers were log2 transformed to approximate a normal distribution. Results were further adjusted for anti-gp350 Ab titers as well as anti-EBV antibody levels, including anti-EBNA1, anti-EBNA2, anti-EA-D, anti-EA-R and anti-VCA.

Results

The association between EBV neutralizing Ab levels and risk of MS was non-significant, though suggestive of an increased risk of MS with increasing antibody titer for individuals with levels measured before disease onset (RR for 1 standard deviation increase in log₂ ng 72A1 equivalent/mL = 2.2, 95% CI: 0.97, 5.1; p = 0.06). Similar results were observed among those with levels measured after onset but before disease diagnosis (RR = 1.7, 95% CI: 0.70, 4.4). Estimates were similar upon further adjustment for anti-EBNA2 Ab titers, anti-EA Ab titers, anti-VCA Ab titers and anti-CMV Ab titers [data not shown]. After adjustment for anti-EBNA1, effect estimates appeared to be attenuated among those with levels measured before disease onset but not in those with blood collected after onset but

prior to disease diagnosis (Table). A similar difference in effect was seen upon adjustment for gp350 Ab titers. For those with blood collected prior to onset, further adjustment for gp350 levels did not affect the estimated association between EBV neutralizing Ab titer and MS risk. In contrast, for those with blood collected after onset but before diagnosis, there was no longer an indication of an association between EBV neutralizing Abs and MS risk after adjustment for gp350 levels (Table).

Discussion

We found only a modest association between EBV neutralizing antibodies and MS risk, which was further attenuated by adjustment for anti-EBNA1 titers. Although not a significant association, contrary to our hypothesis, the direction of effect suggests that if an association exists, it is in the opposite direction of what we had predicted. Given the strong association between a history of EBV infection and subsequent MS risk, it seems logical that those who mount a stronger neutralizing antibody response to EBV would have a lower MS risk. In contrast, higher neutralizing antibody titers were associated with increased (albeit non-significant) MS risk. The fact that these results remained similar upon adjustment for gp350 and anti-EBV antibody titers, suggest it is not merely a reflection of increased EBV presence or activity. These findings, suggest that measurement of EBV neutralizing antibodies is unlikely to contribute substantially to MS risk prediction.

It could be speculated that the mechanism that is regulating the altered immune response that results in MS is also related to EBV neutralizing antibody activity. However, how these are related is not clear, particularly given that neither EBV neutralization *in vivo* nor the autoimmune mechanism resulting in MS is well understood.

There are limitations to the analysis presented here. Foremost, is that our sample size was underpowered to detect modest effects; however, this finding does provide evidence against a strong effect of EBV neutralizing antibodies and MS risk and this is the first report on EBV neutralizing antibodies measured before disease onset and subsequent MS risk. Larger sample sizes are necessary to determine if an association truly exists and further work is necessary to determine the potential role of EBV neutralizing antibodies in protecting against MS.

Acknowledgments

The authors thank Leslie Unger for technical support. This work was funded in part by the National Institute of Health [grants numbers: R01 CA50385, R01 CA67262, P01 CA87969 and R01 CA49449]. The work presented here is the sole content of the authors and does not necessarily represent the view of the National Institutes of Health.

REFERENCES

1. Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. *Ann Neurol*. 2010; 67:824–30. [PubMed: 20517945]
2. Ascherio A, Munger KL. Epstein-Barr Virus Infection and Multiple Sclerosis: A Review. *J Neuroimmune Pharmacol*. 2010; 5:271–7. [PubMed: 20369303]
3. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann Neurol*. 2007; 61:288–99. [PubMed: 17444504]
4. Morgan, AJ.; Khanna, R.; Campadelli-Fumi, G.; Mocarski, E., et al. EBV: Vaccines and Immunotherapy. In: Arvin, AM., editor. *Human Herpesviruses: Biology, Therapy and Immunoprophylaxis*. Cambridge University Press; 2007.
5. Nemerow GR, Mold C, Schwend VK, Tollefson V, Cooper NR. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells:

sequence homology of gp350 and C3 complement fragment C3d. J Virol. 1987; 61:1416–20. [PubMed: 3033269]

6. Sokal EM, Hoppenbrouwers K, Vandermeulen C, et al. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. J Infect Dis. 2007; 196:1749–53. [PubMed: 18190254]
7. Lindsey JW, Hatfield LM and Vu T. Epstein-Barr virus neutralizing and early antigen antibodies in multiple sclerosis. Eur J Neurol. 2010
8. Ascherio A, Munger KL, Lennette ET, et al. Epstein-barr virus antibodies and risk of multiple sclerosis: A prospective study. JAMA. 2001; 286:3083–8. [PubMed: 11754673]
9. Wilson AD, Morgan AJ. Indirect measurement of Epstein-Barr virus neutralising antibodies by ELISA. J Virol Methods. 1998; 73:11–9. [PubMed: 9705170]

Table

Risk of MS associated with increasing levels of neutralizing antibodies to EBV

	<i>Blood draw before onset n=18</i>		<i>Blood draw after onset and before diagnosis n=13</i>	
	RR	95% CI	RR	95% CI
Univariate	2.2	0.97, 5.1	1.7	0.7, 4.4
Adjusted for gp350	2.2	0.8, 6.5	0.5	0.1, 3.6
Adjusted for EBNA1	1.4	0.5, 3.5	1.5	0.5, 5.1

Values presented are for 1 standard deviation increase in the log2 transformed antibody titer levels in ng 72A1 equivalents/mL.