

Schizophrenia is Associated With an Aberrant Immune Response to Epstein–Barr Virus

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Background: Epstein–Barr virus (EBV) is a highly prevalent human herpesvirus capable of infecting the central nervous system and establishing persistent infection. **Methods:** We employed solid phase immunoassay techniques to measure immunoglobulin G (IgG) class antibodies to EBV virions and defined proteins in 432 individuals with schizophrenia and 311 individuals without a history of a psychiatric disorder. Western blot testing was performed to document reactivity to specific EBV proteins. Polygenic risk for schizophrenia was calculated from genome sequencing arrays. Levels of antibodies between the groups were compared by multivariate analyses incorporating clinical, genetic, and demographic measures. **Results:** Individuals with schizophrenia had marked elevations in the levels of antibodies to EBV virions as compared to the control population. Further analyses indicated increased levels of reactivity to EBV-viral capsid antibody (VCA) but not to EBV nuclear antigen-1 (EBNA-1) or to other human herpesviruses. Western blot analysis confirmed increased reactivity to VCA proteins in the group of individuals with schizophrenia and documented a lack of increased levels of antibodies to EBNA-1. Genetic analyses indicated an additive effect of increased levels of antibodies to EBV virions and genetic susceptibility to schizophrenia, with individuals with elevated levels of both type of markers having a greater than 8.5-fold odds of a schizophrenia diagnosis. **Conclusions:** Individuals with schizophrenia have increased levels of antibodies to some but not all EBV proteins indicating an aberrant response to EBV infection. This aberrant response may contribute to the immunopathology of schizophrenia and related disorders.

Key words: schizophrenia/Epstein–Barr virus/herpes virus/infection

Introduction

Schizophrenia is a serious neuropsychiatric disorder of uncertain etiology with a lifetime prevalence of approximately 1% in the United States. While schizophrenia has clear genetic underpinnings, currently known genes explain only a portion of disease risk.^{1,2} In addition to genetic factors, environmental exposures have been identified as increasing risk for the disease. Environmental factors associated with increased risk of schizophrenia include winter-spring birth, urban birth, maternal preeclampsia, and perinatal and postnatal infections.^{3–6} The potential role of infections in the etiopathogenesis of schizophrenia is supported by the associations between schizophrenia risk and genes which encode HLA and other factors which control the immune response to infectious agents.^{1,7}

Epstein–Barr virus (EBV), also known as human herpesvirus 4, is a member of the Herpesviridae family. EBV is a lymphotropic virus that produces latent infections with immunomodulatory effects.^{8,9} Primary infection with EBV is often associated with self-limited fever and adenopathy. Following acute infection, the virus persists in host B and T lymphocytes, monocytes, and epithelial cells; asymptomatic salivary viral shedding leads to onward transmission.¹⁰ EBV can establish latency in many body sites including the brain where reactivation can be associated with encephalitis¹¹ and brain-specific immune responses.¹² The immune response to EBV infection can be monitored by the measurement of levels of antibody directed at antigens derived from virions as well as specific EBV proteins. Commonly measured anti-EBV antibodies include: anti-early antigen (EA) that arises early in the course of infection and decreases after 3–6 months;

viral capsid antibody (VCA) that also arises early in infection but persists for extended periods of time; anti-EB nuclear antigen (EBV NA or EBNA) that does not arise until late in infection but does persist for extended periods of time^{13,14} (supplementary figure 4). The response to additional EBV proteins can be measured by western blotting techniques¹⁵ to further define the immune response to infection.

EBV infections have been associated with a number of autoimmune disorders including multiple sclerosis, systemic lupus, autoimmune encephalitis, and fibromyalgia.^{16,17} In many cases the immune response to EBV in individuals with these disorders is aberrant and differs from the immune response noted in otherwise healthy individuals in terms of the response to EA, VCA, and EBNA antigens.^{18,19}

Many individuals with EBV-associated disorders have psychiatric symptoms during the course of their illness. For example, in the disorder systemic lupus, cognitive dysfunction is reported to occur in more than 80% of patients and psychosis in more than 20%.²⁰ Individuals with multiple sclerosis also have relatively high rates of cognitive impairment²¹ and psychiatric symptoms²² including psychosis.²³ However, there have been few studies of EBV exposure in individuals with schizophrenia. We thus measured the levels of antibodies to EBV virions and defined EBV proteins in a cohort of individuals with schizophrenia and compared these to levels in a group of control individuals without a history of psychiatric symptoms.

Methods

Study Population

The study population consisted of 743 individuals: 432 with a schizophrenia diagnosis and 311 without a history of psychiatric disorder. The details of the recruitment and evaluation of individuals in these populations have been previously described.²⁴ Individuals with schizophrenia met the following criteria: age between 18 and 65 inclusive, diagnosis of schizophrenia or schizoaffective disorder meeting criteria in the Diagnostic and Statistical Manual of Mental Disorder Fourth Edition (DSM-IV); currently receiving antipsychotic medications. Individuals in the nonpsychiatric control sample met the criteria: age between 18 and 60 inclusive, absence of a current or past psychiatric disorder as confirmed by screening with the Structured Clinical Interview for DSM-IV Axis I Disorders – Non-patient Edition (SCID-I/NP). Participants were assessed for the deficit syndrome, a putative schizophrenia subtype comprised of individuals with schizophrenia who have primary and enduring negative symptoms such as restricted affect and diminished social drive.²⁵ Additional details of the methods for recruitment and evaluation are presented in the [supplementary materials and methods](#).

The studies were approved by the Institutional Review Boards of the Sheppard Pratt Health System and the Johns Hopkins Medical Institutions following established guidelines. All participants provided written informed consent after the study procedures were explained.

Antibody Measurements

Immunoglobulin G (IgG) antibodies to EBV antigens derived from intact virions were measured by means of solid phase enzyme immunoassay. Details of the procedure are provided in the [supplementary materials and methods](#). IgG antibodies to EBV-VCA and EBV nuclear antigen-1 (EBNA-1) were measured by solid phase immunoassay employing commercially available assay kits (IBL America).²⁶ IgG antibodies to other human herpesviruses were measured by similar procedures.^{26,27} Complete baseline sample sets consisting of $N = 743$ individual blood samples were available for all immunoassay tests.

Quantitative western blot assays were performed using methods presented in the [supplementary materials](#) and depicted in [supplementary figure 5](#). Samples were available for western blot from 257 individuals, 150 of whom were individuals with schizophrenia and 107 controls. The individuals from whom samples were available for western blot did not differ significantly from the overall study population in any demographic or clinical variable.

Genetic Analyses

DNA was extracted from whole blood and analyzed for genetic polymorphisms using the Illumina array. Polygenic risk score was calculated using a P -value cut-off of $P < .05$ for inclusion of individual polymorphisms used to calculate the polygenic risk score. Details of the methods used are provided in the [supplementary material](#).

Statistical Analyses

The results of the assays were compared between the individuals with schizophrenia and controls employing parametric and nonparametric regression models.²⁸ Details of the statistical methods are presented in the [supplementary material](#). In light of the performance of 3 sets of immunoassay measurements (EBV virions, EBV VCA, EBNA-1), a critical value of $P < .05/3 = .016$ was employed to indicate statistical significance for assays using these measures. A value of $.016 \leq P \leq .05$ was considered to represent a trend.

Results

The demographic and clinical characteristics of the 743 individuals in the study, 432 individuals with schizophrenia and 311 nonpsychiatric controls, are presented in [Table 1](#). Within the schizophrenia group, participants had the following diagnoses per DSM-IV criteria:

Table 1. Demographic and Clinical Characteristics of Study Populations at Initial Assessment^a

| | Schizophrenia (N = 432) | Nonpsychiatric Controls (N = 311) |
|--|-------------------------|-----------------------------------|
| Age (y) | 38.2 (±12.9) | 32.03 (±11.3) |
| Sex male | 284 (65.7%) | 122 (39.2%) |
| Race | | |
| Caucasian | 209 (48.4%) | 153 (49.2%) |
| African American | 208 (48.1%) | 110 (35.4%) |
| Asian | 6 (1%) | 30 (9.6%) |
| Hispanic | 4 (1%) | 4 (1%) |
| Other | 5 (1%) | 5 (1%) |
| Maternal education (y) | 12.9 (±2.6) | 13.8 (±2.5) |
| Years education | 12.3 (±2.2) | 15.5 (±2.5) |
| Body Mass Index | 30.4 (±7.3) | 27.5 (±6.9) |
| Current cigarette smoker | 262 (60.7%) | 46 (14.8%) |
| RBANS cognitive score ^b | 64.7 (±11.7) | 85.5 (±11.9) |
| PANSS total symptom score ^c | 77.7 (±13.9) | — |
| Age of onset (y) | 20.5 (±7.7) | — |
| Duration of illness (y) | 17.4 (±12.3) | — |

Note: ^aContinuous variables expressed as mean (±SD) and dichotomous variables as number (percent). The groups differed significantly ($P < .05$) in variables except race (Caucasian vs non-Caucasian).
^bRBANS, Repeatable Battery for the Assessment of Neuropsychological Status.
^cPANSS, Positive and Negative Syndrome Scale.

schizophreniform disorder ($n = 17$, 4%); paranoid subtype ($n = 51$, 12%); undifferentiated subtype ($n = 126$, 29%); other schizophrenia subtype ($n = 10$, 2%); schizoaffective disorder ($n = 228$, 53%). A total of 124 (29%) of the schizophrenia participants met the criteria for the deficit syndrome. The following antipsychotic medications were the most commonly received at the time of the study assessment by the persons in the schizophrenia group: risperidone ($n = 115$, 27%); olanzapine ($n = 76$, 18%); clozapine ($n = 68$, 16%); ziprasidone ($n = 26$, 6%). The schizophrenia participants also received additional types of psychotropic medications including antidepressants ($n = 169$, 39%) and valproate ($n = 87$, 20%).

Initial analyses were performed to compare the quantitative levels of antibodies between the diagnostic groups. As shown in [figure 1](#) there were significantly elevated levels of IgG antibodies to EBV virions in the schizophrenia group vs the control group (effect size = 0.356; 95% CI 0.168, 0.543; $P < .0002$). This association was confirmed by analysis employing a nonparametric interquartile regression analysis (effect size = 0.467; 95% CI 0.240, 0.693; $P < .0001$). There was also a trend towards increased levels of IgG antibodies to VCA in the schizophrenia group (effect size = 0.197; 95% CI 0.025, 0.370; $P = .025$). However, the levels of antibodies to EBNA-1 did not differ significantly between the groups. Histograms of the distribution of values for these EBV antibodies in individuals with schizophrenia and controls are shown in [supplementary figures 1–3](#) and the prevalence of antibodies in the case and control population are displayed in [supplementary table 1](#). We also measured antibodies to the other human herpesviruses HSV-1, HSV-2, CMV, VZV, and HHV-6. There were no significant differences

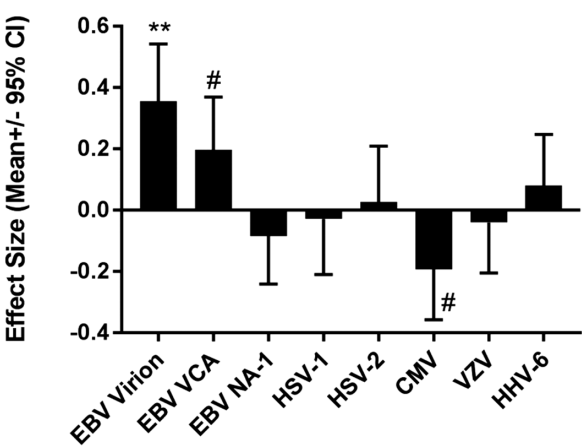


Fig. 1. Effect sizes of immunoglobulin G (IgG) antibody reactivity to Epstein–Barr virus (EBV) and other human herpesviruses by solid phase immunoassays in individuals with schizophrenia as compared to controls calculated using logistic regression models. ** $P < .001$; # $P < .05$.

between the schizophrenia and the control group in any of these antibody levels. There was a trend toward decreased levels of antibodies to CMV in individuals with schizophrenia (effect size = -0.192 ; 95% CI -0.357 , 0.026 ; $P = .023$).

We also examined the odds ratios associated with elevated levels of antibodies defined by values greater than pre-defined percentile levels of the control group adjusted for age, sex, race, cigarette smoking, and maternal education. As depicted in [figure 2](#) we found increased odds of elevated antibodies to EBV virions in the schizophrenia group relative to cutoffs greater than the 50th (OR = 1.71; 95% CI 1.18, 2.48; $P = .005$), the 75th (OR = 2.22; 95% CI

1.50, 3.28; $P < .001$) and the 90th (OR = 2.31; 95% CI 1.39, 3.84; $P = .001$) percentile of the levels of antibodies in the controls. Further, we found a trend to increased odds of antibodies to VCA in the schizophrenia group vs the control group for cutoffs greater than the 50th (OR = 1.45; 95% CI 1.00, 2.08; $P = .048$), and the 75th (OR = 1.54; 95% CI 1.04, 2.28; $P = .032$) percentile, as well as a significant effect at the 90th (OR = 2.03; 95% CI 1.23, 3.37; $P = .007$) percentile of the levels of the antibodies in the controls. There were no significant differences in the odds associated with increased levels of antibodies to EBNA-1.

We further measured the reactivity of samples towards EBV proteins employing a quantitative western blot assay system. As shown in [figure 3](#), there was a significant

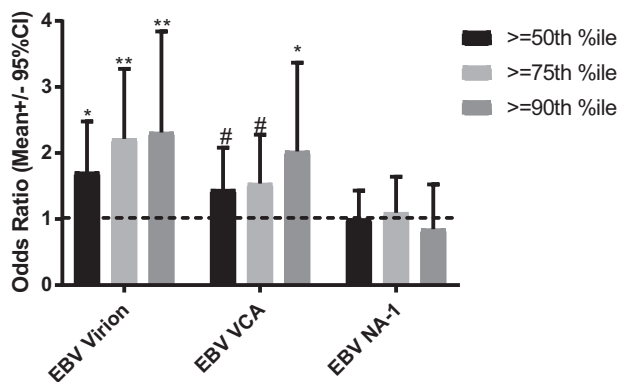


Fig. 2. Odds ratios of immunoglobulin G (IgG) anti-EBV antibody levels in schizophrenia as compared to controls by percentile values of IgG antibodies to Epstein–Barr virus (EBV) virions, EBV Viral Capsid Antigen (VCA) and EBV nuclear antigen (NA). The odds ratios were calculated by the use of logistic regression models ** $P < .001$, * $P < .012$, # $P < .05$.

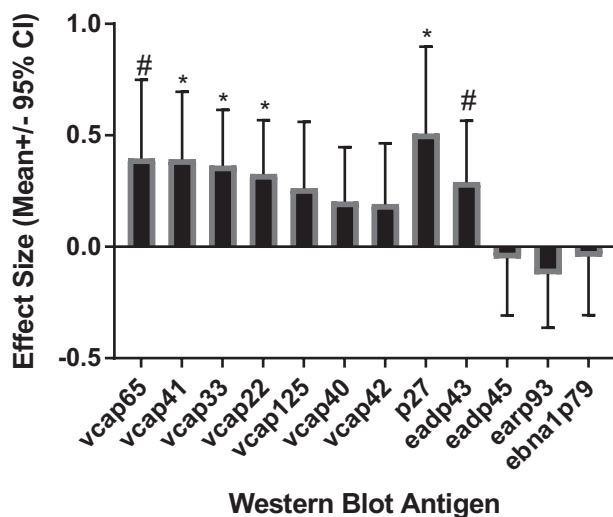


Fig. 3. Effect sizes of immunoglobulin G (IgG) antibody reactivity to individual Epstein–Barr virus (EBV) proteins as measured by quantitative western blot comparing reactivity in individuals with schizophrenia and controls. The effect sizes were calculated using logistic regression models employing * $P < .012$; # $P < .05$.

increase related to schizophrenia diagnosis in the levels of antibodies to VCA p33 (effect size = 0.363; 95% CI 0.113, 0.614; $P < .005$), VCA p22 (effect size = 0.326; 95% CI 0.085, 0.568; $P < .008$), VCA p41 (effect size = 0.392; 95% CI 0.088, 0.696; $P < .012$), and viral protein p27 (effect size = 0.507; 95% CI 0.116, 0.898; $P < .011$). There was also a trend towards increased levels in the schizophrenia group associated with antibodies to VCA p65 (effect size = 0.381; 95% CI 0.033, 0.729; $P = .032$) as well as the early antigen EA-D p43 (effect size = 0.290; 95% CI 0.015, 0.565; $P < .039$). There were no schizophrenia-associated increases in antibodies to the other antigens including EBNA-1 p79 and the other early antigens.

We examined the bivariate relationship between antibodies to EBV virions and clinical and demographic variables within the group of individuals with schizophrenia. In terms of basic demographic variables, the levels of antibodies to EBV virions were positively associated with increased age (correlation coefficient = .023; 95% CI .020, .038; $P < .0001$), female sex ($F = 9.93$, $P < .0017$), lower levels of maternal education (correlation coefficient = $-.070$; 95% CI $-.115$, .024; $P < .003$), cigarette smoking ($F = 8.31$, $P < .0042$) and non-Caucasian race ($F = 15.75$, $P < .0001$) but not with participant educational level, age of onset, illness duration, or birth outside of the United States or Canada (all $P > .1$).

We employed regression models to examine the relationship between levels of antibodies to EBV virions and clinical variables as described in the [supplementary methods](#) employing age, sex, race, cigarette smoking and maternal education. We found that levels of antibodies to EBV virions were significantly associated with the presence of deficit syndrome (effect size = 0.363; 95% CI 0.111, 0.616; $P < .005$) and the administration of the medication valproate (effect size = 0.399; 95% CI 0.119, 0.680; $P < .005$). There were no significant associations with the PANSS symptom score, RBANS cognitive score, BMI, or other medications (all $P > .05$).

We also examined the interaction between EBV antibodies and the genetic risk for schizophrenia as measured by the polygenic risk score employing regression models adjusted for age, sex, race, cigarette smoking, and maternal education. The polygenic risk score was associated with an increased risk of schizophrenia in the study population (effect size = 0.498; 95% CI 0.245, 0.751; $P < .001$) but was not significantly associated with the level of anti-EBV virion, anti-EBV VCA, or anti-EBNA-1 antibodies ($P > .1$). Analyzed in terms of percentiles, a polygenic risk score of ≥ 50 th percentile was associated with a schizophrenia diagnosis with an odds ratio of 2.18 (95% CI 1.27, 3.74; $P < .005$) and a polygenic risk score of ≥ 75 th percentile was associated with a schizophrenia diagnosis with an odds ratio of 1.61 (95% CI 0.904, 2.88; $P > .1$).

There was an apparent additive effect of odds ratios associated with the polygenic risk score and EBV virion

antibodies. The odds ratio for schizophrenia diagnosis associated with having both EBV virion antibody levels and the schizophrenia polygenic risk score ≥ 50 th percentile was 3.41 (95% CI 1.47, 7.95; $P < .004$ adjusted for age, sex, race, maternal education, cigarette smoking, and genotyping array). The odds ratio for schizophrenia diagnosis associated with having both EBV virion antibody levels and the schizophrenia polygenic risk score greater than the 75th percentile was 8.86 (95% CI 2.59, 30.37; $P < .001$). There was no significant statistical interaction between the levels of EBV virion antibody and the polygenic risk score in relation to their association with schizophrenia ($P > .1$).

Follow-up samples were obtained and analyzed from 183 individuals: 131 individuals with schizophrenia and 52 controls. The median interval between the initial and follow-up was 182 days (interquartile range 112–578 d). The individual levels of EBV antibodies to virions VCA and EBNA-1 at the first and last sampling periods were highly correlated (effect size = 0.88, 0.76, and 0.84, respectively). None of the differences between the levels measured at the first and second sampling were statistically significant ($P > .05$ adjusted for age, sex, race, diagnosis, maternal education and time interval between first and second sampling).

Discussion

We found that individuals with schizophrenia have increased levels of IgG antibodies to EBV virions as compared to a control group. The differences were independent of demographic variables that are known to affect EBV exposure such as age, sex, race, and socioeconomic status as measured by maternal education. We also measured antibodies to specific viral proteins. These studies indicated that the individuals with schizophrenia had an aberrant immune response to EBV in that there were elevated levels of antibodies to EBV VCA but not to EBNA-1. It is likely that most of the participants in our study have undergone primary EBV infection at some time in the past as evidenced by the presence of low levels of antibody to EBV early antigen (EA). Generally, individuals with past infections to EBV have increased levels of IgG class antibodies to both VCA and EBNA-1 antigens.¹³ The levels of EBV antibodies in the study populations are relatively stable over extended periods of time since the levels did not change significantly in the individuals for whom follow-up samples were available. This finding suggests that the aberrant immune response is unlikely to be related to timing of sampling but is relatively stable within an individual over time; additional longitudinal studies should be done to assess the stability of the markers over time. Antibodies to other herpesviruses including HSV-1, HSV-2, CMV, VZV, and HHV-6 were not significantly increased in the same population of individuals with schizophrenia. This finding indicates

that the increased levels of antibodies to EBV virions found in the individuals are not reflective of a heightened immune response to human herpesvirus but is specific to EBV within this group of infectious agents.

As noted above, healthy individuals generally develop and maintain approximately equal levels of antibodies to both EBV VCA and EBNA proteins following the resolution of acute infection. The finding of increased levels of VCA but not EBNA-1 antibodies in individuals with schizophrenia is thus reflective of an aberrant immune response to EBV infection. Aberrant responses to EBV have been described in a range of EBV-associated disorders including autoimmune diseases such as multiple sclerosis²⁹ and systemic lupus as well as neoplastic conditions such as nasopharyngeal carcinoma.³⁰

Increased levels of antibodies to EBV virion and VCA proteins were not associated with cognitive functioning or symptom scores but were associated with an increased prevalence of the deficit syndrome. EBV virion antibodies were also significantly increased in individuals who were receiving the mood-stabilizing medication valproate. This finding, which should be verified in larger samples, is of interest in light of the immunomodulatory effects of this medication which are likely related to its effects on histone deacetylation.³¹ Valproate has also been used in the treatment of EBV-associated tumors based on its ability to modulate EBV gene expression.³¹ Increased levels of EBV antibodies were also marginally associated with an increased prevalence of cigarette smoking.

The pattern of reactivity to EBV proteins in the study cohort was further analyzed by performing western blot analyses directed at IgG antibodies directed at 12 EBV proteins. These analyses confirmed significant reactivity to VCA proteins and other EBV proteins as well as the lack of reactivity to EBNA-1. Of interest is the significant increased reactivity to p27 in individuals with schizophrenia as compared to controls. The material provided by the manufacturer of the quantitative immunoblot system that we employed states that the viral strain used to produce the blot strips is P3HR1. Although we have not yet confirmed the identity of this 27kDa protein present on the blot strips, it is likely that this p27 represents the truncated form of the EBNA leader protein that Garibal et al³² reported to be produced by P3HR1 EBV strain but not the predominant, wild-type strains, suggesting that individuals with schizophrenia might have been differentially exposed to an EBV variant with a mutation similar to this strain.

Primary EBV infection generally occurs in adolescence following viral transmission facilitated by oral contact. Infection in adolescents is often manifested by a syndrome of fever, pharyngitis, lymphadenopathy, and splenomegaly generally referred to as infectious mononucleosis. Most cases of infectious mononucleosis are self-limited and are followed by increases in antibodies to VCA and EBNA proteins. The timing of primary EBV

infection is of interest in terms of the first manifestations of schizophrenia which also often occur in adolescence. It is of note in this regard that Khandaker et al found that previous exposure to EBV as measured by VCA antibodies was associated with subsequent psychotic experiences in adolescence.³³

Our studies indicate that the source of EBV antigen in the test immunoassay is important in evaluating the association between EBV exposure and a schizophrenia diagnosis. It is thus of interest that DeWitte et al did not find an association between levels of antibodies to EBV and a schizophrenia diagnosis.³⁴ However, as they measured EBNA antibodies (DeWitte L, personal communication) as markers of EBV exposure, their findings are consistent with ours. The consistency of our results with other studies that have measured EBV antibodies in individuals with schizophrenia^{35–37} is difficult to evaluate without additional information relating to the immune responses to defined EBV proteins.

The reasons for altered levels of antibodies to EBV proteins and specific EBV proteins are not known with certainty. Possible mechanisms for this association include ones relating to the virus and to the host response. In terms of the virus, a differential response to infection might be related to variation in the timing of primary EBV exposure, the genomic composition and pathogenicity of the infecting EBV, and possible re-exposure to differing strains of EBV.³⁸ Virological analysis of samples obtained from individuals in prospective studies will be required to address these possibilities. An altered immune response to EBV infection could also be based on host factors such as genetics or other environmental factors. In terms of genetic factors, we found an additive effect of increased EBV antibodies and increased genetic risk with a combined odds ratio of 8.86 for individuals with values ≥ 75 th percentile of both measures. However, there was no statistically significant association interaction between the levels of EBV virion antibodies and the polygenic risk scores suggesting that the risks associated with genetic susceptibility and increased levels of EBV virion antibodies are independent. However larger sample sizes might be employed to detect low levels of interaction. Furthermore, additional genetic studies are required to define the genetic contribution to the aberrant response to EBV infection found in individuals with schizophrenia. The possibility use of combinations of immune and genetic markers for the diagnosis and management of schizophrenia is an important area for future study. Antigen-specific antibodies to additional herpesviruses and other viruses might also be examined with the goal of incorporating them into this approach.

In terms of other environmental factors, it is of note that we found an association between levels of antibodies to EBV virion proteins and cigarette smoking in individuals with schizophrenia. An interaction between EBV exposure and cigarette smoking has also been noted in

other EBV-associated disorders such as multiple sclerosis, possibly based on the many immunomodulatory effects of cigarette smoking.³⁹ As for the above factors, the timing of the interaction between cigarette smoking and EBV exposure in terms of risk for schizophrenia should be addressed in prospective studies. The possible effects of lymphotropic viruses such as EBV and other environmental factors which can affect B-cell activity on the immunopathology of schizophrenia should be the subject of future investigations.

The neurobiological mechanisms by which increased levels of EBV virion antibodies might be associated with schizophrenia are not entirely known. One potential scenario is that individuals with aberrant EBV immune responses underwent previous replication of EBV within the central nervous system. This possibility is consistent with reports of psychosis in individuals undergoing EBV encephalitis and the finding of increased levels of EBV VCA antibodies in the CSF of some individuals with psychiatric disorders.^{40–45} It is also possible that psychiatric symptoms are related to neuro-inflammatory effects on the brain including alterations in neurotransmitter receptor interactions, as has been found in a range of autoimmune disorders which affect the brain.⁴⁶

In conclusion, this study indicates that many individuals with schizophrenia have an aberrant immune response to EBV. There are a number of therapeutic interventions available for the modulation of EBV infection including anti-viral medications and pharmacological compounds which can modulate the immune response.⁴⁷ An increased understanding of the role of EBV infection might thus lead to novel methods for the prevention and treatment of schizophrenia.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

Funding

NIMH P50 Silvio O. Conte Center at Johns Hopkins (MH-94268); Stanley Medical Research Institute (07-1690 to F.D.).

Acknowledgment

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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