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Distinct Epstein–Barr Virus Antibody Profiles in Schizophrenia, Bipolar Disorder, and Major Depressive Disorder: Implications for Potential Therapies

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Abstract

Increasing evidence implicates immune dysregulation in the pathophysiology of serious psychiatric disorders. Epstein–Barr virus (EBV) establishes lifelong infection and elicits complex immune responses which can be associated with neuropsychiatric abnormalities. We employed the recently developed technique of largescale immunoprecipitation sequencing to characterize protein-specific antibody responses to EBV proteins in individuals with schizophrenia, bipolar disorder, and major depressive disorder (MDD), including individuals with MDD and a recent suicide attempt. Differential antibody reactivity to EBV peptides was assessed employing regression models adjusted for age, sex, race, cigarette smoking, and multiple comparisons We identified 16 distinct EBV proteins with significantly altered reactivity in at least one psychiatric group. Individuals with schizophrenia exhibited the greatest number of differences, characterized by increased antibodies to several early lytic and latency II/III proteins (including EBNA-2, BLRF2, BFRF3, and LMP-1) and decreased antibodies to EBNA-1 and EBNA-3 proteins. Bipolar disorder was marked by selective suppression of EBNA-1 and EBNA-3A. Individuals with MDD showed broad suppression of latency-associated antibodies, accompanied by increased antibodies to the late lytic protein BPLF. Individuals with MDD and a recent suicide attempt displayed marked suppression of EBNA-LP1, a protein critical to B-cell transformation. In contrast to EBV, antibody responses to other human herpesviruses showed few associations with diagnosis. Several psychiatric disorders are associated with distinct patterns of antibody responses to EBV proteins reflecting differential regulation of viral latency and lytic activity. These findings support a role for EBV-related immune mechanisms in psychiatric illness and highlight potential avenues for targeted antiviral or immunomodulatory interventions.

Introduction

Schizophrenia, bipolar disorder, and major depressive disorder affect over 5% of the global population and represent leading causes of disability and mortality worldwide. (1) Converging evidence implicates inflammation as a central pathophysiological mechanism in these disorders, particularly in the context of genetic susceptibility. (2–4) However, the specific factors that trigger and sustain inflammatory processes have not been well characterized.

Recent studies have indicated that some individuals with serious psychiatric disorders have an altered immune response to Epstein–Barr virus (EBV). (5, 6) This virus, also known as human herpesvirus 4, is a gammaherpesvirus with near-universal worldwide distribution. Primary EBV infection typically occurs during childhood or adolescence and is often asymptomatic, although it may manifest as infectious mononucleosis, a clinical syndrome characterized by fever, pharyngitis, and lymphadenopathy. Following acute infection, which is generally self-limited, EBV establishes lifelong persistence in host B lymphocytes and can also be detected in T lymphocytes, monocytes, and epithelial cells. EBV can establish latency in multiple tissues, including the central nervous system, where viral reactivation has been associated with encephalitis, neoplastic disorders, and autoimmune responses. (7–11)

The systemic immune response to EBV infection is commonly assessed through measurement of antibodies directed against virion components as well as specific viral proteins expressed during lytic infection or latency. Frequently measured anti-EBV antibodies include antibodies to the early antigen (EA), which arise early during infection and typically decline within 3–6 months; antibodies to the viral capsid antigen (VCA), which also appear early but persist for extended periods; and antibodies to Epstein–Barr nuclear antigen 1 (EBNA-1), which generally emerge later in infection and remain detectable lifelong. Measurement of these antibody profiles is widely used to monitor primary infection, distinguish acute from past infection, and infer viral reactivation.

Alterations in these canonical antibody patterns have been described in several disease states characterized by dysregulated immune responses to EBV. Such altered profiles are observed in EBV-associated malignancies as well as immune-mediated disorders, including multiple sclerosis, systemic lupus erythematosus, scleroderma, and fibromyalgia. (12) (13–15) Among these conditions, the association between EBV and multiple sclerosis has been most extensively studied. In this disorder, early-life EBV exposure appears to be a necessary antecedent, with disease development thought to reflect impaired T-cell–mediated control of viral replication.(16) This impaired control, influenced by both genetic and environmental factors, is accompanied by markedly elevated antibody responses to EBNA-1 and other EBV antigens. (13)

Recent studies have also documented an aberrant immune response to EBV in individuals with psychiatric disorders. For example, we have previously reported an altered EBV antibody profile in individuals with schizophrenia, characterized by increased levels of antibodies to EBV VCA in the absence of a corresponding increase in antibodies to EBNA-1. (13) Elevated VCA antibody levels were additionally associated with increased genetic risk for schizophrenia, as assessed by polygenic risk scores. In this population, increased EBV antibody levels were further linked to higher hazards of natural-cause mortality (17) and to impaired cognitive functioning.(18) Associations between EBV antibodies and schizophrenia have been reported by other investigators as well. Moreover, increased EBV antibody levels have been detected in the cerebrospinal fluid of some individuals with schizophrenia spectrum disorders relative to serum levels, suggesting possible central nervous system involvement. (19) (20) Childhood EBV exposure has also been associated with an increased risk of psychotic experiences later in life. (21)

Evidence for an altered immune response to EBV has also been observed in major depressive disorder. In contrast to schizophrenia, individuals with MDD demonstrated no increase in antibodies to EBV VCA but exhibited elevated antibodies to intact virions along with a marked decrease in antibodies to EBNA-1. (22) Support for a relationship between EBV and depression is provided by Mendelian randomization studies suggesting shared genetic architecture, (6) as well as population-based investigations indicating that childhood EBV infection manifesting as infectious mononucleosis is associated with an approximately 40% increased hazard of a subsequent diagnosis of depression. (23) Nonetheless, other studies have reported null associations between EBV and depression, underscoring the heterogeneity of findings. (24)

It is of note, however, that commonly used methods for EBV serological profiling capture only a small portion of the immune response to this complex virus. EBV's large DNA genome encodes more than 90 proteins expressed across different stages of infection and latency, each capable of eliciting distinct immune responses. (9) Previous studies have been limited by the number of EBV proteins that could be targeted in a practical manner. However, the recent development of high-throughput immune profiling technologies (25) (26) now enables comprehensive characterization of antibody responses to the full EBV proteome, potentially revealing disorder-specific immune signatures not detectable by conventional methods. We hypothesized that individuals with psychiatric disorders would differ from those without psychiatric disorders in terms of the immune response to defined EBV proteins. To address this hypothesis, we applied one of the previously reported high-throughput techniques, phage display immunoprecipitation sequencing (PhIP-Seq) (24, 27) to measure antibodies directed against EBV peptides in blood samples from individuals with schizophrenia, bipolar disorder, and major depressive disorder, including a subset of individuals with major depressive disorder who had recently attempted suicide. Antibody profiles were compared with those obtained from a cohort of control individuals without a history of psychiatric illness. These profiles were used to propose pathways relating to viral latency, reactivation, and replication.

Methods

Study populations

Study participants included individuals with serious mental illness (schizophrenia, bipolar disorder, or major depressive disorder) as well as a comparison control group consisting of individuals without a history of a psychiatric disorder. All were enrolled through the Stanley Research Program at Sheppard Pratt, a large nonprofit psychiatric health system in Baltimore, Maryland, USA. The details of participant enrollment and evaluation have been previously described (28–31) (32, 33) and are summarized as follows.

The psychiatric participants were recruited from inpatient and outpatient psychiatric programs at Sheppard Pratt. Persons in the non-psychiatric comparison group were recruited from posted announcements at Sheppard Pratt and other local hospitals. All participants were screened for diagnostic eligibility with the Structured Clinical Interview for Diagnosis (SCID), the patient or non-patient version. (34) Inclusion criteria for all the participant groups included: age 18–65 years (except the first study which was 20–60 years of age); capacity to provide written informed consent; proficient in English.

Exclusion criteria for all the participant groups included: history of IV drug use; HIV + or other immunodeficiency condition; serious medical condition that affects brain or cognitive functioning; diagnosis of intellectual disability or a history of severe learning disorder.

Additional inclusion criteria for the study of persons with schizophrenia or bipolar disorder included: primary diagnosis (DSM-IV-TR) of bipolar disorder; schizophrenia or schizoaffective disorder. Additional

exclusion criteria for this study included: diagnosis of substance abuse or dependence according to DSM-IV-TR criteria within the last 3 months; taking antiviral medications in the past one week.

Additional inclusion criteria for individuals with MDD were a current diagnosis of major depressive disorder as defined by DSM-5 and a current admission to a Sheppard Pratt inpatient or day hospital program for a major depressive episode. The individuals with MDD were selected to be included in one of two groups: persons who had a suicide attempt in the past 30 days, or persons who did not have any suicide attempt history or suicidal ideation within the past 30 days. The occurrence of a suicide attempt (aborted, interrupted, or actual) and the presence of suicidal ideation were based on the criteria of the Columbia Suicide Severity Rating Scale. (35)

Additional exclusion criteria for the study of persons with MDD included: diagnosis of substance abuse or dependence according to DSM-5 criteria within the last 3 months; presence of underlying celiac disease, Crohn's disease, inflammatory bowel disease, or other systemic disorder known to be associated with persistently elevated levels of markers of gastrointestinal inflammation; use of a potent systemic anti-inflammatory agent in the past 2 weeks.

Additional exclusion criteria for individuals in the non-psychiatric comparison group were: current or past psychiatric disorder or treatment for a mental health condition.

All of the studies were approved by the IRBs of Sheppard Pratt and the Johns Hopkins School of Medicine. All participants provided written informed consent following explanation of the study procedures.

At the study visit, participants were interviewed to obtain demographic information including age, sex, self-reported race, and whether the person was a current tobacco smoker. Participants were also asked about their place of birth (foreign or not) and their mother's level of education as a proxy for socioeconomic status. All participants had a blood sample drawn on the day of the study visit. The plasma from the samples was separated by centrifugation and stored at -70 degrees until testing.

Analytic Methods

Blood samples were analyzed using viral PhIP-Seq antibody profiling as previously described(36). The library contains > 480,000 oligonucleotides encoding 62-mer amino acid peptides with 14-mer overlaps, covering most vertebrate, mosquito-borne, and tick-borne viral proteins to 90% identity

Aliquots of the phage library were reacted with 0,2 µL of plasma (20 µl of a 1:100 dilution) from the study participants and were immunoprecipitated with Protein A/G-coated magnetic beads alongside mock-immunoprecipitation controls. Following PCR incorporation of multiplex barcodes and sequencing adapters, samples underwent paired-end Illumina sequencing to > 4× input library complexity. Paired-end reads were demultiplexed, trimmed, and aligned using bwa mem.

The edgeR exactTest function (37) was employed to estimate fold changes and P values for each sample versus mock immunoprecipitations. Positive reactions required log2 fold change ≥ 2.0 (> 4 fold increase over beads-only controls) and unadjusted $P < 10^{-4}$. Data were mapped to 87,601 peptides with known human tropism, then to individual EBV proteins via sequence annotations and alignments.

For each peptide, the association with the diagnostic categories was examined by regression models employing age, gender, race, and current tobacco smoking as covariates. These covariates were selected based on previous studies of demographic factors associated with EBV antibodies. (38) Significance levels between the diagnostic groups and the control group were determined by Wald tests.

For each EBV protein, we selected the peptide with the minimum p value for further analysis to avoid inflating significance through multiple testing of overlapping peptides from the same protein. The resulting p values were adjusted for multiple comparisons using the false discovery method of Benjamini et al(39), with an adjusted p level (q value) < 0.1 considered statistically significant. The reactivities to the peptides which showed a significant response were further expressed as the mean difference in reactivity as compared to the control group consisting of individuals with no history of a psychiatric disorder. For each diagnostic group, plausible pathways were constructed using known biological properties of the EBV proteins which were the targets of the dysregulated antibodies.

We further performed sensitivity analyses to define the specificity of the reactions to EBV. These were performed by the application of the same protocol to the measurement of reactivity in the sample set to the additional human herpesviruses: Herpes Simplex Virus Type 1 (HSV-1, Human Herpesvirus 1, HHV-1). Herpes Simplex Virus Type 2 (HSV-2, Human Herpesvirus 2, HHV-2), Varicella Zoster Virus (VZV, Human Herpesvirus 3) HHV-3), Cytomegalovirus (CMV, Human Herpesvirus 5, HHV-5), Human Herpesvirus 6 (HHV-6), Human Herpesvirus 7(HHV-7), and Human Herpesvirus 8. (HHV-8).

Software

All statistical analyses were performed using STATA Version 19.5 (STATA Corporation, College Station Texas, USA. Pathway diagrams were created with BioRender Software, Toronto, Ontario, Canada.

Results

Table 1 presents the demographic and clinical characteristics of study participants. The five groups (N = 100 total) did not differ significantly in age, race, place of birth, or maternal education. Individuals with schizophrenia had a higher proportion of males (40.6% vs 9.4–18.8% in other groups) and current tobacco smokers (28.6% vs 0–5.0% in other groups

Table 1
Demographic and Clinical Characteristics of Study Participants

Variable	Controls	Schizophrenia	Bipolar Disorder	MDD-No Suicide	MDD-Recent Suicide Attempt
Number	20	21	21	20	18
Age, years (mean)	40.4	37.0	34.7	37.1	37.8
Maternal education, years (mean)	13.5	13.2	14.4	13.5	13.3
Sex, male (%)*	15.6	40.6	9.4	15.6	18.8
Race, White (%)	45.0	52.4	57.1	30.0	22.2
Tobacco smoker (%)**	5.0	28.6	0.0	5.0	0.0*
Birth, United States (%)	90.0	100.0	95.2	90.0	88.9

*Abbreviations: MDD, major depressive disorder; ** p<.01, *p<.05 among the groups by two-way analysis of variance.*

Table 2

A. Epstein-Barr Virus Proteins Showing Differential Reactivity to a Diagnostic Group Compared to the Comparison Control Group

EBV Gene	Gene Name	Role in Latency/Lytic Infection	Protein Function
BALF2	Single-stranded DNA-binding protein	Early lytic	DNA polymerase processivity factor
BARF1	Secreted decoy CSF1 receptor	Latency/Early lytic	Immunomodulatory functions
BDLF2	Tegument protein/membrane protein	Late lytic	DNA-binding protein
BFRF3	Small capsid protein (sCP)	Late lytic	Viral capsid protein
BLLF1	Major glycoprotein gp350/220	Late lytic	Viral attachment to B cells
BLRF2	Tegument protein	Late lytic	Virion assembly
BORF2	Ribonucleotide reductase large subunit	Early lytic	Nucleotide metabolism
BPLF1	Large tegument protein/deubiquitinase	Late lytic	Modulates immune responses
BZLF1	Immediate early transactivator (Zta)	Immediate early lytic	Master regulator of lytic cycle
EBNA-1	Epstein-Barr Nuclear Antigen 1	All latency programs	Viral episome maintenance
EBNA-2	Epstein-Barr Nuclear Antigen 2	Latency III	B-cell transformation
EBNA-3A	Epstein-Barr Nuclear Antigen 3A	Latency III	Transcriptional regulator
EBNA-3B	Epstein-Barr Nuclear Antigen 3B	Latency III	Modulates immune recognition
EBNA-3C	Epstein-Barr Nuclear Antigen 3C	Latency III	Cell cycle regulation
EBNA-LP	Epstein-Barr Nuclear Antigen Leader Protein	Latency III	Coactivates EBNA-2
LMP-1	Latent Membrane Protein 1	Latency II, III	Oncogenic signaling

Abbreviations: *EBV, Epstein-Barr virus; EBNA, Epstein-Barr nuclear antigen; LMP, latent membrane protein. The reactivity of the individuals in the diagnostic groups to the EBV proteins is depicted in* Figs. 1 and 2.

Table 2B. Epstein-Barr Virus Proteins Not Showing Significant Differences Among Diagnostic Groups

EBV Gene	Gene Name	Role	Protein Function
BALF4	gp110 (gB glycoprotein B)	Lytic	Viral fusion and entry
BALF5	DNA polymerase catalytic subunit	Lytic	DNA synthesis
BBLF1	Tegument protein	Lytic	Virion assembly
BBLF4	Helicase	Lytic	DNA replication
BBRF1	BamHI-B rightward frame 1	Lytic	Portal vertex protein
BCLF1	Major capsid protein (MCP)	Lytic	Capsid shell
BCRF1	Viral IL-10	Lytic	Immune evasion
BDLF1	VP19 homolog (capsid)	Lytic	Capsid assembly
BFLF1/BFLF2	Bcl-2 homolog/Unknown	Lytic	Apoptosis inhibition
BGLF1	Alkaline nuclease	Lytic	DNA processing
BGRF1	BamHI-G rightward frame 1	Lytic	Unknown
BHLF1	Bcl-2 homolog	Lytic	Apoptosis inhibition
BILF2	GP78/55	Lytic	Tegument protein
BKRF4	Tegument protein	Lytic	Virion structure
BLLF3	BamHI-L leftward frame 3	Lytic	Unknown
BMLF1	SM protein (p40)	Lytic	Post-transcriptional regulator
BVRF2	p40 (ICP27 homolog)	Lytic	Virion assembly
BMRF1	Early Antigen Diffuse (EA-D)	Lytic	DNA polymerase processivity
BOLF1	Tegument protein	Lytic	Virion maturation
BRLF1	Rta (R transactivator)	Lytic	Early lytic gene activation
BRRFΩ	BamHI-R rightward frame Omega	Lytic	Unknown
BSRF1	BamHI S rightward reading frame 1	Lytic	Tegument protein
BVRF1/BVRF2	Tegument proteins	Lytic	Virion structure
BWRF1	EBNA-LP exon	Latency	Part of EBNA-LP
BXLF1	Thymidine kinase	Lytic	Nucleotide metabolism
BXLF2	gH (gp85)	Lytic	Viral fusion and entry
BZLF2	gp42	Lytic	Binds MHC class II
FGAM	FGAM-synthase	Lytic	Purine biosynthesis

LF2	RJ1 LF2/BILF4	Lytic	Inhibits viral replication
LMP2A	Latent Membrane Protein 2A	Latency	BCR signaling mimic
MAG	ATPase subunit of terminase	Lytic	DNA packaging
Uncharacterized	Unknown	Unknown	Function unknown

Abbreviations: EBV, Epstein-Barr virus; EBNA, Epstein-Barr nuclear antigen; IL-10, interleukin-10; LMP, latent membrane protein; MCP, major capsid protein.

Of 87,601 peptides in the library, 13,316 showed at least one positive reaction with a blood sample, including 667 peptides derived from EBV. These EBV peptides mapped to 49 viral proteins spanning latent and lytic phases of infection (Tables 2A and 2B).

We compared EBV antibody profiles across four psychiatric groups (schizophrenia, bipolar disorder, MDD without recent suicide attempt, MDD with recent suicide attempt) versus non-psychiatric controls using regression models adjusted for age, sex, race, and cigarette smoking. After false discovery rate correction, 16 EBV proteins showed differential antibody reactivity in at least one psychiatric group ($q < 0.1$; Figs. 1–2).

All psychiatric groups showed decreased antibodies to EBNA-1, EBNA-3B, and BORF2 compared to the controls. However, substantial differences distinguished the diagnostic groups (Fig. 1).

Schizophrenia exhibited the most extensive immune alterations, with elevated antibodies to seven early lytic proteins (BLRF2, EBNA-2, BLLF1, BFRF3, LMP-1, BALF2, BARF1) not observed in other groups. Individuals with schizophrenia also showed decreased antibodies to late lytic proteins and additional latency-associated proteins (EBNA-3A, EBNA-3C).

Bipolar disorder was characterized primarily by suppressed antibodies to latency-associated proteins (EBNA-3A, EBNA-3C, and the three proteins decreased across all groups), with elevated EBNA-3B antibodies suggesting shifted CD8 + T-cell epitope recognition.

Major depressive disorder without a recent suicide attempt showed elevated antibodies to BPLF1 (a late lytic deubiquitinase) not found in other diagnostic groups, alongside decreased antibodies to latency-associated proteins.

Major depressive disorder with a recent suicide attempt differed from MDD without a suicide attempt history through additional suppression of EBNA-LP antibodies, a finding not observed in any other diagnostic category. This diagnostic group also showed decreased EBNA-3A, EBNA-3C, and BDLF2 antibodies, patterns shared with schizophrenia and bipolar disorder.

Functional pathway analysis (Fig. 3A-D) revealed distinct patterns of latency and lytic infection dysregulation across diagnostic groups. These pathways are described in detail in the accompanying

figure legends.

To assess EBV specificity, we examined antibody responses to seven other human herpesviruses (HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, HHV-8) across all diagnostic groups (Table 3). No proteins from HSV-1, CMV, VZV, HHV-6, or HHV-7 showed differential reactivity in any psychiatric group (all q > 0.1). A single HSV-2 tegument protein showed increased reactivity in MDD without recent suicide attempt.

Table 3
Reactivity of Samples to Other Herpesviruses

Herpesvirus	Number of Peptides with Reactivity	Number of Represented Proteins	Scz	Bip Dis	MDD-No Suicide Attempt	MDD-Recent Suicide Attempt
HHV-1/HSV-1	395	59	0	0	0	0
HHV-2/HSV-2	251	47	1	0	0	0
VZV	141	35	0	0	0	0
CMV	618	127	0	0	0	0
HHV-6	263	78	0	0	0	0
HHV-7	123	38	0	0	0	0
HHV-8	232	55	0	0	0	0

Values under the diagnostic groups indicate the number of peptides with significantly increased reactivity compared to the comparison control group. Abbreviations: Scz, schizophrenia Bip Dis, bipolar disorder; CMV, cytomegalovirus; HHV, human herpesvirus; HSV, herpes simplex virus; MDD, major depressive disorder; VZV, varicella zoster virus.

Discussion

Using comprehensive immune profiling of EBV-directed antibodies, we identified distinct, disorder-specific immune signatures in schizophrenia, bipolar disorder, and major depressive disorder. These findings extend prior observations of aberrant EBV immunity in psychiatric disorders by revealing that different disorders exhibit fundamentally different patterns of viral-immune dysregulation. Importantly, these alterations were largely specific to EBV, with minimal differences in antibody responses to seven other common human herpesviruses, suggesting selective impairment of immune control of EBV infection across psychiatric disorders.

Our results demonstrate that psychiatric disorders are not immunologically homogeneous regarding EBV but rather exhibit differences in terms of the immune response to EBV. Our results also suggest distinct underlying pathophysiological mechanisms relating to EBV replication highlighting ongoing viral reactivation in schizophrenia as opposed to immune exhaustion to latency antigens in the mood disorders.

Aberrant immune responses to EBV have been found in several neoplastic and autoimmune disorders. Among these disorders the response has been most extensively characterized in multiple sclerosis where antibodies to EBV appear to be a necessary first step in the development of the disorder. (40) Although multiple sclerosis differs from the psychiatric disorders studied here in terms of neurological symptoms and brain pathology, it is often associated with a range of psychiatric symptoms (41). These psychiatric symptoms include psychosis which can occur prior to the onset of neurological symptoms. (42)

The schizophrenia antibody profile shares some features with multiple sclerosis while differing in critical ways. Both conditions show elevated EBNA-2 antibodies; EBNA-2 is a master regulator of B-cell transformation that interacts with CD40, a key autoimmunity mediator. This parallel suggests shared mechanisms of EBV-driven immune dysregulation. (43) It is of note that CD40 has been linked to a decreased risk of schizophrenia through genetic studies and Mendelian randomization analyses. (44) (45)

However, schizophrenia and multiple sclerosis diverge in terms of EBNA-1 responses. Elevated EBNA-1 antibodies are a cardinal feature of multiple sclerosis and likely drive molecular mimicry-mediated autoimmunity to myelin. (46) In contrast, EBNA-1 antibodies are suppressed in schizophrenia and other psychiatric disorders studied here, suggesting immune exhaustion rather than hyperreactivity to latency antigens.

These immunological differences align with HLA genetic architecture. HLA-DRB1*15:01 increases multiple sclerosis risk (47) but shows protective effects against schizophrenia; HLA-DRB1*01:01 shows the opposite pattern. (48) Since HLA genotypes strongly influence EBNA-1 antibody levels, HLA variation may explain the divergent EBNA-1 responses and the absence of multiple sclerosis-like demyelinating pathology in schizophrenia despite shared EBNA-2 reactivity.

In terms of the other psychiatric disorders examined in our study, the immune response to EBV in individuals with bipolar disorder or major depressive disorder was largely characterized by a decrease in antibodies to EBV proteins which control latency. In the case of bipolar disorder similarly to schizophrenia, antibodies to EBNA-3A are the most strongly suppressed; suppression of this gene is associated with growth arrest following initial proliferation during latency IIb and III. In contrast, antibodies to EBNA-3B are elevated. Increased recognition of EBNA-3B with concurrent suppression of EBNA-3C may reflect a shift in $CD8^+$ T-cell epitope recognition. (49)

Individuals with major depressive disorder also displayed decreased levels of antibodies to EBNA-1 and other EBV proteins involved in latency. However, individuals with this disorder also displayed an increase in the levels of antibodies to BPLF1 not found in the other diagnostic groups. BPLF1 is a late lytic gene that encodes a large tegument deubiquitinase (DUB) that inhibits innate immune signaling by blocking IRF3, TRAF6, and NEMO, while also modulating NF-κB signaling. Elevated antibodies to BPLF1 may indicate increased abundance of this DUB, which would be expected to disrupt multiple downstream pathways regulating host inflammatory gene expression. (50)

It is of note that individuals with major depressive disorder who had undergone a recent suicide attempt had further differences from those individuals with this disorder who did not have a history of suicide attempts. Most striking is a decrease in antibodies to EBNA-Leader Protein (EBNA-LP1), a difference not found in the individuals with major depressive disorder who did not have a recent suicide attempt or any of the other psychiatric groups. EBNA-LP1 is the first latent protein expressed after primary infection and is critical for B-cell transformation. (51) These findings are of interest in light of studies indicating an association between apoptosis in blood mononuclear cells and an increased risk of suicide in individuals with major depressive disorder. (52)

The disorder-specific antibody patterns we observed suggest distinct viral-immune mechanisms across psychiatric diagnoses. In schizophrenia, elevated early lytic antibodies with suppressed late lytic antibodies indicate aborted lytic reactivation. a state in which cells enter the lytic cycle but fail to complete viral replication with repeated cycles of incomplete reactivation potentially driving chronic inflammation and neuroinflammation. The finding of recurrent EBV lytic activity and B-cell dysregulation suggests that interventions directed at the inhibition of EBV replication might be explored in clinical trials for schizophrenia. Of particular interest are recent reports that tenofovir, an antiviral widely used for HIV pre-exposure prophylaxis, inhibits EBV replication in vitro.(53) Notably, tenofovir is currently under investigation in clinical trials for multiple sclerosis, another condition with an established EBV link, raising the prospect of similar trials in schizophrenia.(54) On the other hand, the patterns of EBV immune response in individuals with bipolar disorder or major depressive disorder suggest a focus on immune-based interventions targeting B-cell dysregulation and T-cell exhaustion, as has been proposed for other EBV-associated disorders.(10)

Study Limitations

There are several limitations to our study. First, the number of individuals in each diagnostic group was relatively modest, raising the possibility that additional differences in the immune response to EBV among the groups might become evident in larger samples. In addition, the cross-sectional nature of our study precludes analysis of changes in the immune response to EBV over time. Prospective studies would be valuable for defining the temporal association between EBV infection, the immune response, and psychiatric symptoms. A related methodological constraint is that the immunological method employed uses peptides based on linear epitopes and therefore cannot identify immune responses to antigenic sites formed by conformational epitopes as have been noted for other herpesviruses. (55) Furthermore, our study focused on antibody measurements and did not assess T-cell responses to EBV, which play critical roles in viral control. Given the central role of cytotoxic T-cell responses in EBV immune surveillance, future studies integrating T-cell profiling with antibody data will be essential for a complete picture of EBV immune dysregulation in psychiatric populations. It should also be noted that we were unable to fully control for potential effects of psychiatric medications including antipsychotics, mood stabilizers, and antidepressants which may themselves influence antibody profiles and represent a potential confound. Finally, while our sample is demographically representative of the clinical population

at our institution, replication in more diverse populations is needed to confirm the generalizability of these findings.

Conclusions

Individuals with schizophrenia, bipolar disorder, and major depressive disorder exhibit distinct EBV antibody profiles. These disorder-specific immune signatures suggest different underlying pathophysiological mechanisms relating to the immune response to EBV infection. The divergent immune profiles across diagnoses, particularly the evidence of aborted lytic reactivation in schizophrenia, suggest that antiviral and immunomodulatory strategies may need to be tailored to specific diagnostic contexts rather than applied uniformly across psychiatric conditions. Future research should examine whether these antibody profiles predict treatment response, clinical trajectory, or relapse risk, and whether they can guide the selection of immunomodulatory or antiviral interventions. As precision psychiatry advances, EBV immune profiling may emerge as a tool for identifying biologically defined subgroups amenable to targeted therapeutic strategies.

Declarations

Conflict of Interest Statement

The authors have no conflicts to disclose.

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Figures

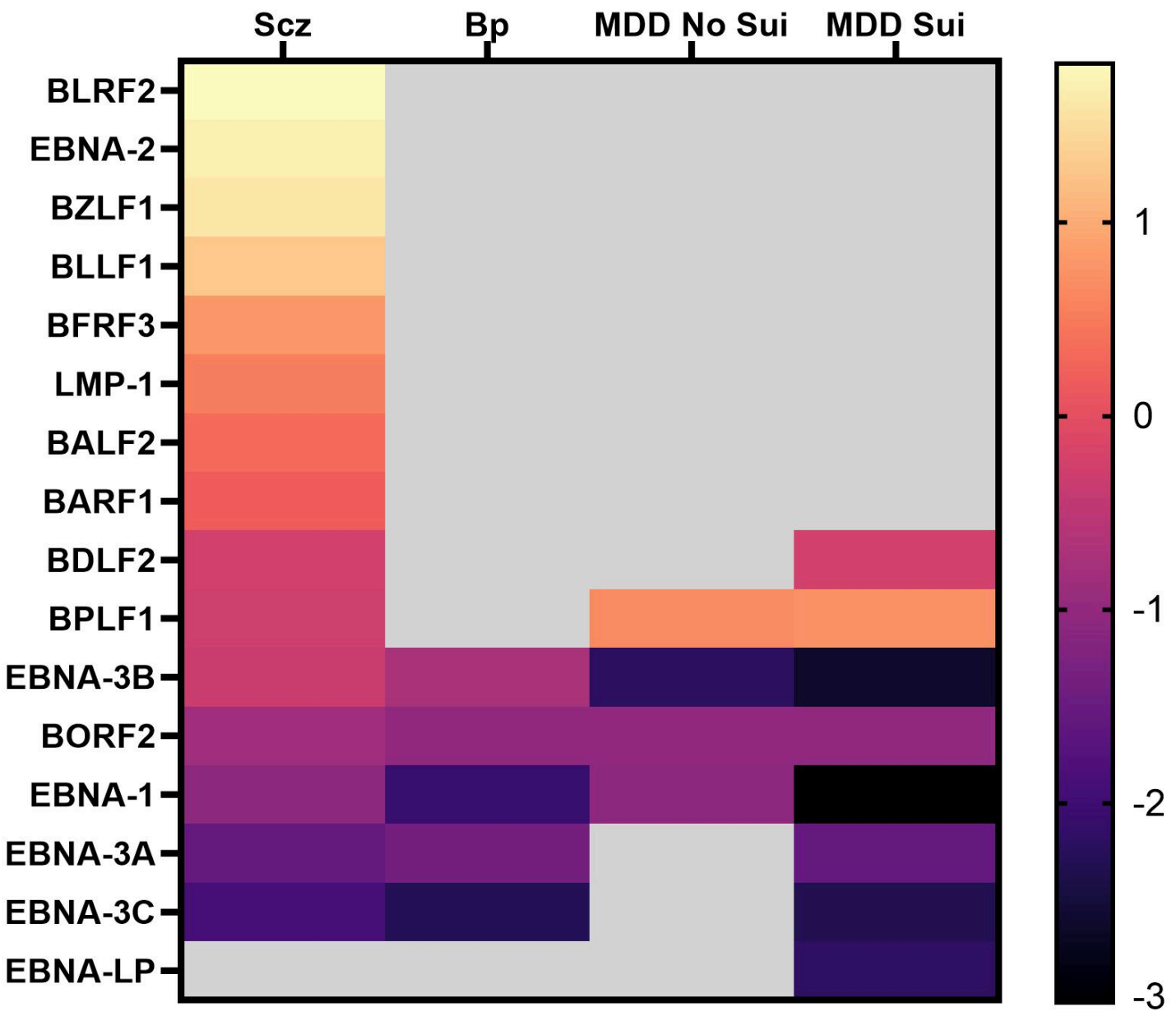


Figure 1

Relative levels of antibodies to EBV proteins in individuals with psychiatric disorders compared to individuals without a psychiatric disorder. The colors represent mean differences measured as described in the text. All of the levels in the colored bars are significantly different from the control group as measured by regression models as described in the text, q<.1 adjusted for false discovery. The full names of the EBV proteins are depicted in Table 2A along with the corresponding phase of viral latency of replication and the peptide which was analyzed.

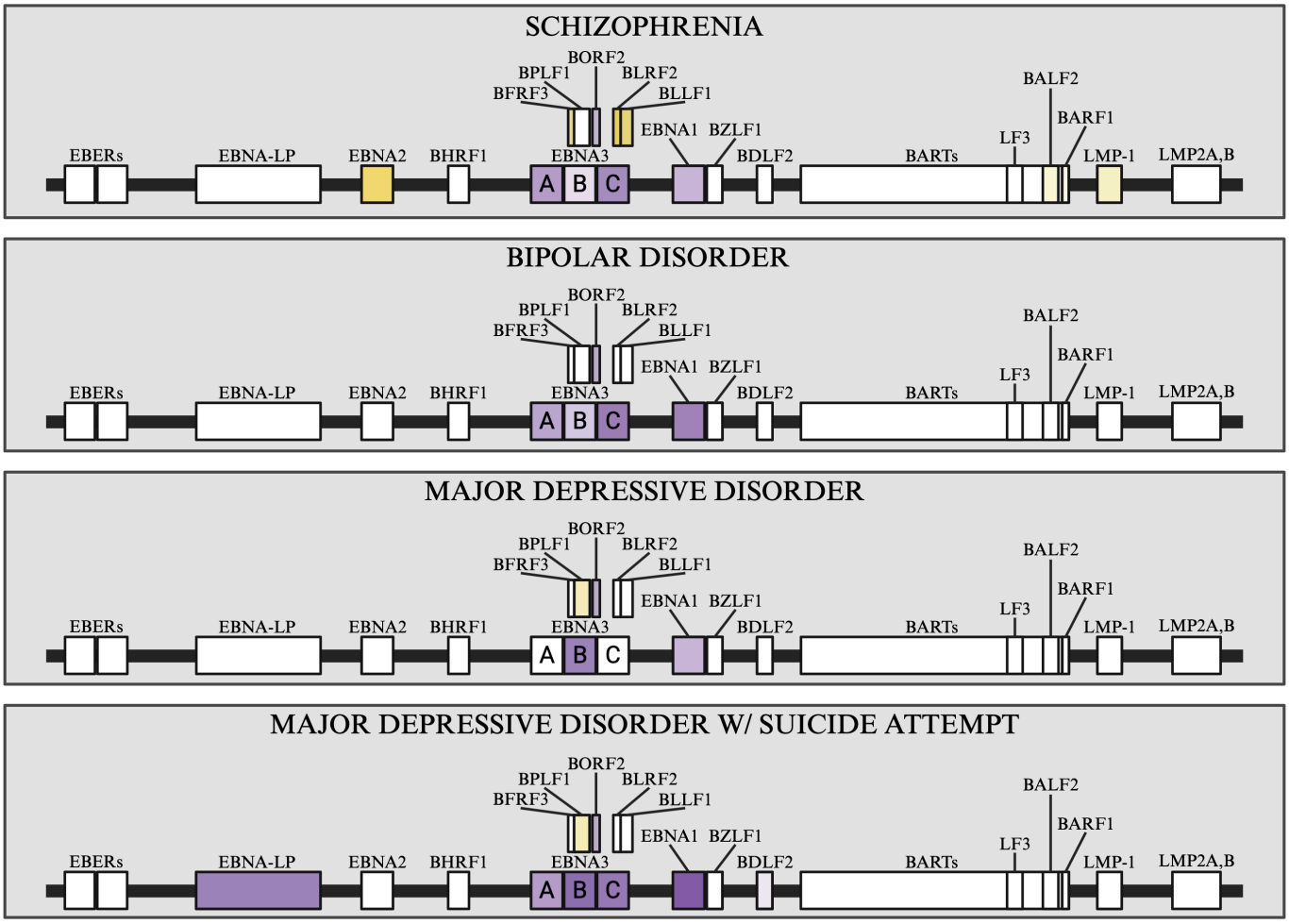


Figure 2

Genetic maps of EBV proteins targets which differed between individuals in the diagnostic groups and the control comparison group. The color scale is the same as the one employed in Figure 1. The full names of the EBV proteins are depicted in Table 2A along with the corresponding phase of viral latency of replication and the peptide which was analyzed.

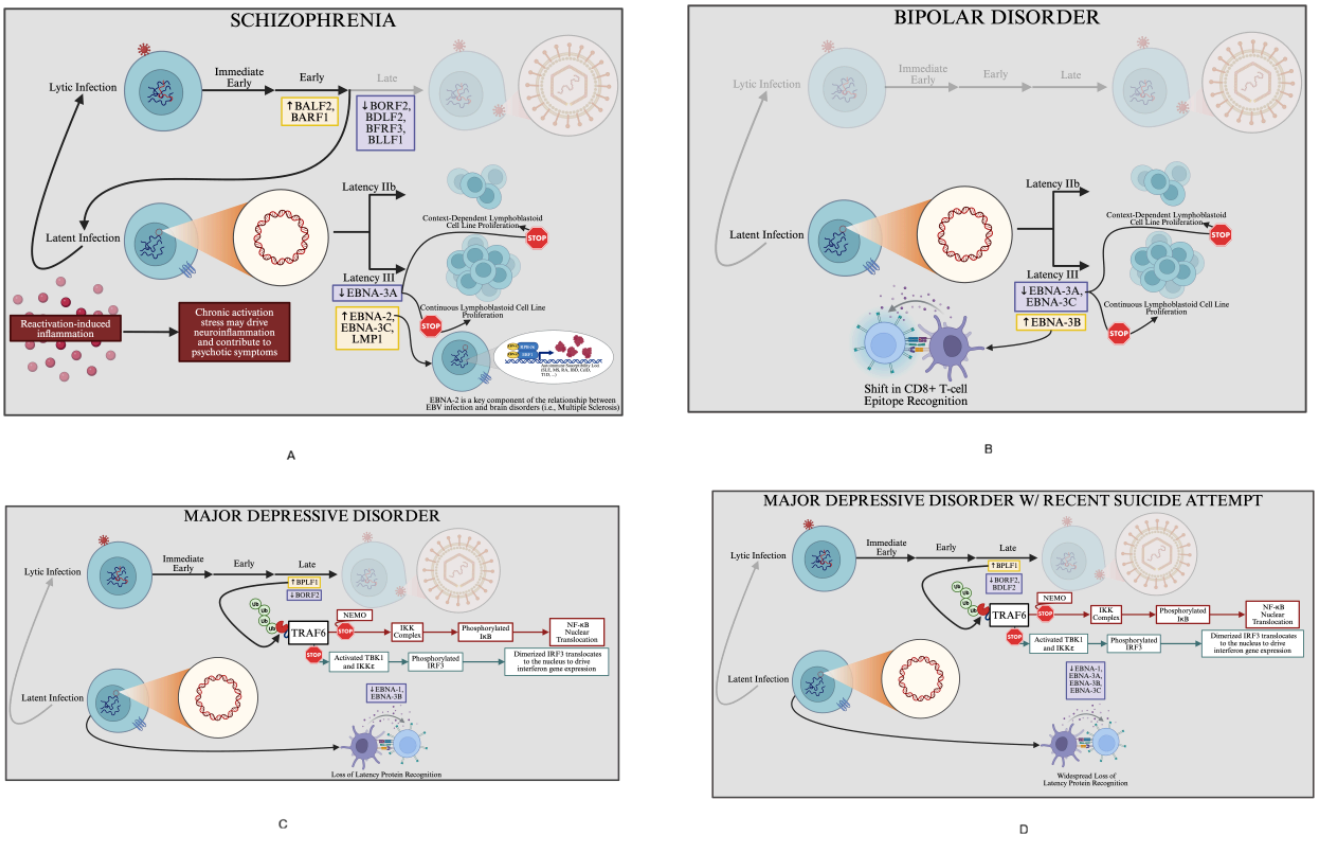


Figure 3

Functional pathways consistent with the EBV proteins which differed between individuals in the diagnostic groups and the controls

A **Schizophrenia** - Antibodies to early lytic genes are elevated, whereas antibodies to late lytic genes are suppressed. Cells that are unable to progress to the late lytic phase may return to latency. Repeated cycling between latent and lytic states may lead to chronic inflammation that contributes to neuroinflammation. Antibodies to genes associated with latency IIb and latency III are elevated, both of which are latency programs that promote lymphoblastoid cell line proliferation. However, antibodies to EBNA-3A are the most strongly suppressed; suppression of this gene is associated with growth arrest following initial proliferation. EBNA-2, a gene associated with latency IIb and III, has also been linked to autoimmune conditions such as multiple sclerosis through its binding of DNA-binding proteins at autoimmune susceptibility loci.

B. Bipolar Disorder – Antibodies to EBNA-3A are the most strongly suppressed; suppression of this gene is associated with growth arrest following initial proliferation during latency IIb and III. In contrast, antibodies to EBNA-3B are elevated. Increased recognition of EBNA-3B with concurrent suppression of EBNA-3C may reflect a shift in CD8⁺ T-cell epitope recognition.

C. Major Depressive Disorder - Individuals without a history of a suicide attempt - BPLF1 is a late lytic gene that encodes a large tegument deubiquitinase (DUB) that inhibits innate immune signaling by blocking IRF3, TRAF6, and NEMO, while also modulating NF-κB signaling. Elevated antibodies to BPLF1 indicate increased abundance of this DUB, which would be expected to disrupt multiple downstream pathways regulating host inflammatory gene expression. In addition, suppression of antibodies to BORF2, which is required for viral DNA replication, indicates that the normal lytic cascade is not being followed. Antibodies to two latency-associated genes, EBNA-1 and EBNA-3B, are also suppressed. EBNA-1 is expressed across latency I, IIa, IIb, and III; suppression of antibodies to this gene suggests emerging immune exhaustion or tolerance to latency antigens.

D. Major Depressive Disorder - Individuals with a recent suicide attempt. Antibodies to BPLF1 are elevated, indicating increased abundance of this DUB and disruption of downstream inflammatory regulatory pathways. Again, suppression of antibodies to BORF2 suggests that the normal lytic cascade is not being followed. Unique to MDD with a recent suicide attempt is suppression of BDLF2, another late lytic gene, which further indicates that the normal lytic cascade is not being followed. Examination of latent infection markers reveals substantial suppression of EBNA-1 and pan-EBNA-3 family antibodies. This degree of suppression indicates loss of immune recognition of core latency antigens.