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HYPOTHESIS

Abortive lytic Epstein–Barr virus replication in tonsil-B lymphocytes in infectious mononucleosis and a subset of the chronic fatigue syndrome

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Abstract: A systematic 2001–2007 review of 142 chronic fatigue syndrome (CFS) patients identified 106 CFS patients with elevated serum IgG antibodies to the herpesviruses Epstein–Barr virus (EBV), cytomegalovirus, or human herpesvirus (HHV) 6 in single or multiple infections, with no other co-infections detected. We named these 106 patients group-A CFS. Eighty-six of these 106 group-A CFS patients (81%) had elevated EBV early antibody, early antigen (diffuse), serum titers. A small group of six patients in the group-A EBV subset of CFS, additionally, had repetitive elevated-serum titers of antibody to the early lytic replication-encoded proteins, EBV dUTPase, and EBV DNA polymerase. The presence of these serum antibodies to EBV dUTPase and EBV DNA polymerase indicated EBV abortive lytic replication in these 6 CFS patients. None of 20 random control people (age- and sex-matched, with blood drawn at a commercial laboratory) had elevated serum titers of antibody to EBV dUTPase or EBV DNA polymerase ($P < 0.01$). This finding needs verification in a larger group of EBV CFS subset patients, but if corroborated, it may represent a molecular marker for diagnosing the EBV subset of CFS. We review evidence that EBV abortive lytic replication with unassembled viral proteins in the blood may be the same in infectious mononucleosis (IM) and a subset of CFS. EBV-abortive lytic replication in tonsil plasma cells is dominant in IM. No complete lytic virion is in the blood of IM or CFS patients. Complications of CFS and IM include cardiomyopathy and encephalopathy. Circulating abortive lytic-encoded EBV proteins (eg, EBV dUTPase, EBV DNA polymerase, and others) may be common to IM and CFS. The intensity and duration of the circulating EBV-encoded proteins might differentiate the IM and EBV subsets of CFS. Abortive lytic replication may be a pathogenic mechanism for EBV disease. EBV (HHV4) is a gamma herpesvirus composed of dsDNA about 170 Kb in length. For this discussion, there are early genes (including expressions of encoded proteins EBV dUTPase, DNA polymerase, and nuclear proteins) and late genes (including expressions of capsid and membrane proteins). Abortive infection infers incomplete virion expressions of either early or late proteins, but the virion is incomplete. The lytic virus infers a complete virion. The pathologic consequences of EBV abortive replication are currently being investigated by authors.

Keywords: Epstein–Barr virus, chronic fatigue syndrome, subset chronic fatigue syndrome, abortive lytic replication

Background

Chronic fatigue syndrome (CFS) is an orphan and a mystery.[1–8] CFS patients and CFS physician-scientists are sometimes isolated by patients' families, friends, and other physicians who consider CFS a psychiatric condition. We have pursued pathologic physiology[9–12] and reported tachycardias at rest,[4] abnormal oscillating T-wave

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electrical repolarizations with flat abnormal and ischemic-like T-waves, and, ultimately, cardiac dilatation with an associated, diminished left-ventricular ejection fraction. We found there to be primary cardiomyopathy at cardiac biopsy.[5,6] In three peer-reviewed publications, including a placebo-controlled trial and a recent systematic review, we report longlasting clinical improvement with valacyclovir in Epstein–Barr virus (EBV) subset of CFS patients.[1,12–14] Kogelnik et al have reported similar therapeutic results in human herpesvirus (HHV) 6/EBV subset group-A CFS with valganciclovir,[8] and Jason et al estimate there are 800,000 CFS patients in the USA.[15,16] CFS remains a conundrum.

EBV is a virtually universal human infection that is a primary infection in the tonsils with two target cells: epithelial cells and naïve B-lymphocytes. EBV is also a tumor-associated virus expressing latent EBV replication in Hodgkin's disease, nasopharyngeal carcinoma, Burkitt lymphoma, and gastric carcinoma.[17–23] EBV infection in the tonsillar ring of Waldeyer is associated with a soft exudate in infectious mononucleosis (IM),[24] and there is an unrecognized pathognomonic, hard white exudate in some patients with the EBV subset of CFS (Lerner, unpublished data, 2002). Remarkably, even in acute IM, there is no complete virion infectious virus in the blood.[3] Remarkably, too, a low level of EBV lytic replication continues in B-lymphocytes, in the tonsils, which have differentiated into plasma cells during the entire lifetime of every individual.[25,26] After primary EBV infection in the tonsils, naïve B-lymphocytes in the tonsil differentiate into activated blasts and express the growth transcription programs EBNA 1, 2, 3A, 3C, LP, LMP1, and LMP2. These activated EBV-infected B-lymphocytes migrate to an adjacent oligoclonal germinal center, where they differentiate into memory B-cells[27–29] (Figure 1). Within memory B-cells, EBV is a closed, non-replicating intranuclear episome expressing only EBVNA1 and selected viral RNAs, which, when present, in turn allow the memory B-cell to divide and therefore expand its population while still in the periphery. According to Miyashita et al,[27] memory B-cells safely leave the tonsil to enter the peripheral circulation expressing no detectable EBV complete virion. Memory B-cells in the circulation are safe from EBV cytolytic T-lymphocytes, which recognize mainly structural EBV-encoded proteins.[30–33] EBV episome-carrying memory B-cells are long-lived and permanent residents in the periphery. Thorley-Lawson[33] further posits that EBV-memory B-cells intermittently reenter the tonsils. In the tonsils, again, these EBV memory B-cells differentiate into plasma cells and replicate, again encoding EBV lytic proteins. These EBV lytic replicating plasma cells now may die by apoptosis, freeing complete virions into the saliva (Figure 1).[30–36]

EBV major envelope glycoprotein gp/350/220 binds to naïve B-lymphocyte membrane protein C3d complement receptor 2 (CR2/CD21) to infect virtually the entire human population.[24,37,38] An elevated serum IgG antibody to the structural protein EBV viral capsid antigen (VCA) with a concomitant negative IgM VCA EBV serum antibody records past infection.[39] Nevertheless, healthy people intermittently shed EBV infectious virus in their saliva.[30,31]

Quantitative studies of EBV replication during IM indicate lytic replication in the tonsils is in the minority, and abortive lytic replication is dominant.[25,31,32] During acute IM, immune/inflammatory responses of both cell-mediated and humoral systems target multiple antigens, including latent and lytic-encoded proteins. Encoded proteins EBNA 1, 2, 3A, 3B, 3C, LMP1, and LMP2, and the early lytic cycle antigens, including the encoded genes BZLF1 (Zta), BRLF1 (Rta), and early antigen diffuse component (EA[D]) BMLF1, elicit responses. In IM, MHC cytolytic T-cell antigenic responses promptly free the periphery of lytic virus.[30,31] Some healthy EBV immune carriers may have low titers of circulating serum antibody to EBV early antigen (diffuse) (EA[D]), which express early EBV epitopes 86–100 and 268–277.[18,26,35,37]

Recent findings

Diagnostic panel and group-A and -B subsets

In order to define a laboratory-based EBV subset of CFS, we name CFS patients with elevated serum EBV EA(D) BMRF1 serum titers of antibody EBV subset group A.[1] Other clinically identical group-A CFS patients have elevated serum IgG antibody titers to beta herpesviruses, for example, cytomegalovirus (CMV) or HHV6 in single or multiple infections, but no other co-infections.[1] We describe a laboratory-based diagnostic panel, which assures a CFS homogenous group of patients. Previous CFS studies have been limited or based upon symptoms only.[40–52] In our CFS systematic review,[1] we found 36 additional so-called CFS patients who had serologic evidence of herpesviruses infection (EBV, CMV, HHV6) and one or more co-infections. Co-infections were tick-borne *Borrelia burgdorferi*, *Babesia microti*, *Anaplasma (Ehrlichia) phagocytophilum*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, or infection associated with rheumatic fever. From this systematic review,

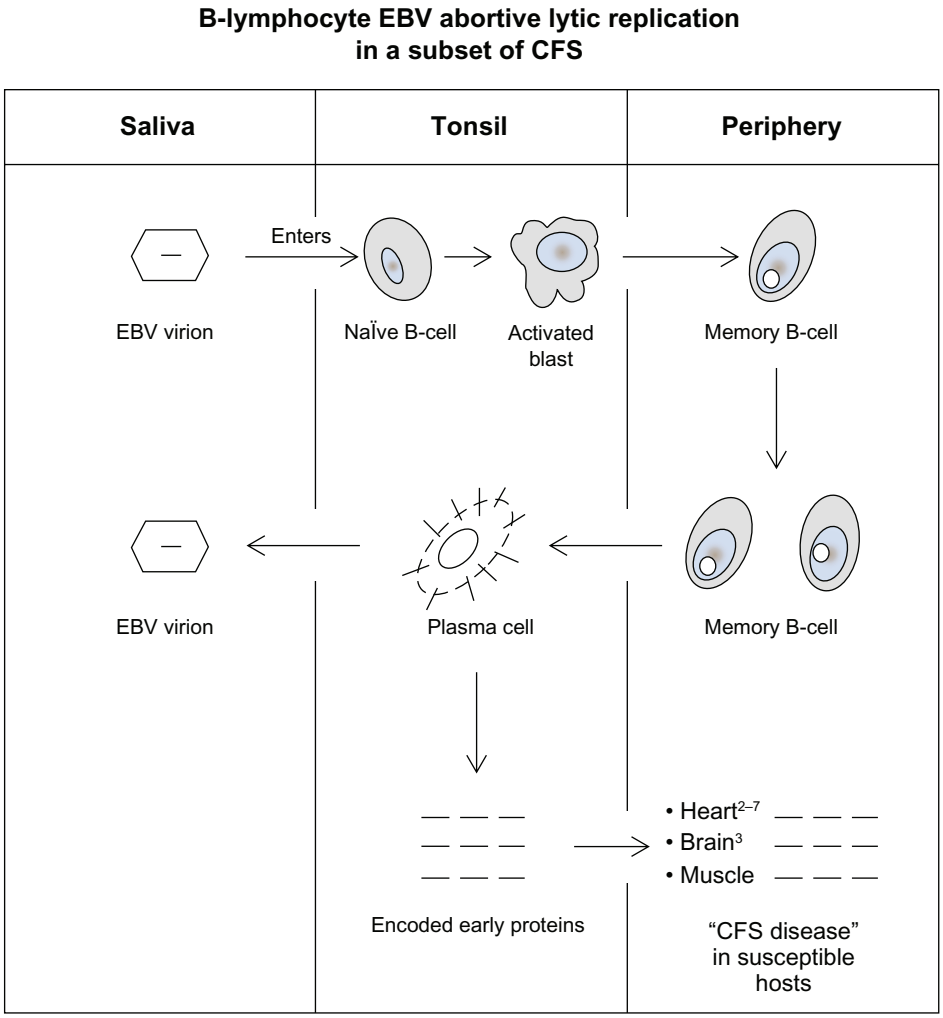

Figure 1 B-lymphocyte EBV abortive lytic replication in a subset of CFS.

Notes: Complete EBV virion is transferred by saliva (kisses) from an asymptomatic EBV carrier to primarily infect epithelial cells in the tonsils of the recipient, where lytic replication (complete virions) occurs. In turn, naïve B-lymphocytes of the recipient are infected. These B-lymphocytes differentiate into activated blasts in the tonsils. The activated blast B-lymphocytes then enter the periphery and become memory-B cells containing intranuclear EBV latent virus.[28,29] Ultimately, the memory-B cells reenter the tonsils where again some of these memory B-cells differentiate into plasma cells. Again in the tonsils, plasma cell apoptosis with modest lytic replication, but marked abortive lytic replication ensues, producing the encoded proteins EBV DNA polymerase and EBV dUTPase, among others. These encoded proteins in CFS (and IM) enter the periphery, "producing" disease (myocarditis, encephalopathy, myositis, hepatitis, etc, in IM).[4]

Abbreviations: CFS, chronic fatigue syndrome; EBV, Epstein–Barr virus; IM, infectious mononucleosis.

we named the 36 additional CFS patients with co-infections group-B CFS.[1]

Long-term CFS group and subset-directed EBV pharmacokinetic antiviral treatment

Accepted canonical science interprets an absence of EBV VCA IgM antibody and the absence of EBV DNA-emia as signifying no active infection.[39] The in vitro and animal model studies of Glaser et al[53,54] suggest abortive lytic replication may be critical to IM and CFS; short-term acyclovir therapy has been unsuccessful.[52] This CFS diagnostic panel (used here) was not available in earlier studies.[1, 40–52] The group-A CFS subset includes patients with EBV, CMV, and HHV6 infection, but with no other detected co-infections. Group-B CFS patients include EBV, CMV, and HHV6 infections with co-infections. EBV subset group-A CFS patients were treated with valacyclovir, and with valganciclovir only if elevated serum IgG antibody to CMV or HHV6 were also present. Valacyclovir (14.3 mg/kg every [q] 6 hours) with valganciclovir (two, 450 mg q 24 hours) was given for up to 12 months. (Valganciclovir was given q 12 hours as needed.[1]) Of the 106 group-A patients, 79 recovered to live normal lives (74.5%, $P < 0.0001$).[1] There was no EBV VCA IgM serum

antibody in 90% of cases and no free EBV DNA-emia in the EBV subset of CFS. The EBV genome is latent in memory B-cells in the periphery and no lytic virus is in the blood.[33] This dosage of valacyclovir and the duration of valacyclovir therapy distinguish our valacyclovir use from earlier trials of acyclovir by others, which failed to reverse the course of CFS. Valacyclovir is the prodrug of acyclovir. Acyclovir binds to EBV DNA, leading to irreversible viral inactivation and interruption of lytic replication at the level of EBV DNA polymerase.[12] EBV latent replication is not interrupted.[39] Valacyclovir levels in serum at this dosage (14.3 mg/kg q 6 hours) reach anti-EBV inhibitory concentrations of 22 μm (IC_{50} EBV, 4.4–13.3 μm).[13] To our knowledge, these results have not been previously reported. Clinical improvement began only after sixth months of valacyclovir treatment, and continued through 12 months. Accepted canonical science, however, interprets the absence of EBV VCA IgM antibody, and the absence of EBV DNA-emia, as not being EBV lytic replication.[39,55]

Latest findings

Lerner et al[2] report on 6 of 19 (32%) randomly selected people with low-titer elevated-serum antibody to the nonstructural encoded proteins EBV EA(D), BMRF1.[2] In contrast, 86 of 106 (81%) CFS patients in the 2001–2007 systematic review had elevated serum titers of antibody to the encoded EBV EA(D) proteins.[1] At the 10th IACFS Conference for Physicians and Healthcare Professionals Translating Evidence into Practice (Ottawa, Canada, September 22–25, 2011), Lerner et al[2] presented six group-A EBV subset of CFS patients who had elevated serum antibody to the encoded proteins EBV EA (D) and elevated serum-neutralizing antibody to the encoded proteins EBV DNA polymerase and EBV dUTPase.[2] Elevated serum antibody to EBV DNA polymerase and EBV dUTPase persisted for at least 400 days. None of 20 controls had elevated serum antibody to the encoded proteins EBV DNA polymerase or EBV dUTPase ($P < 0.01$). These group-A EBV subsets of CFS patients had mean serum-neutralizing titers of antibody to EBV DNA polymerase of 60 u/mL, compared to the mean 17 u/mL ($P < 0.01$) of control titers. The six EBV group-A CFS patients demonstrates "EBV abortive lytic replication."[2]

Miller et al,[56] Jones et al,[57] Liu et al,[58] and Natelson et al[59] have all reported elevated serum antibody values for both EBV DNA polymerase and EBV dUTPase in several CFS patients, but many CFS patients did not have these findings in each of the earlier studies. The classification of CFS patients to the group-A EBV subset of CFS made possible the recognition of this uniform group of CFS patients. This uniformity was achieved because of the diagnostic panel we reported, and its subsequent recognition of the group-A EBV subset.[1]

It is possible that elevated serum antibody to EBV dUTPase and EBV DNA polymerase separates EBV CFS subset patients from healthy immune carriers (Table 1).[2] In vitro, encoded dUTPase[53] and EBV-encoded Zta induce cellular dysregulation and apoptosis.[60] Unassembled EBV-encoded early proteins, including among others, EBV DNA polymerase and EBV dUTPase, may enter the periphery in both the IM and EBV subsets of CFS. We postulate that these circulating EBV-encoded abortive lytic proteins attach to cellular membranes and enter multiple organs, including the heart (cardiomyopathy),[6,24] muscle (myositis), brain, and meninges (encephalopathy),[8] and subsequently incite an explosive inflammatory response-producing disease.[61] Symptoms and pathologic findings in the IM and EBV subsets of CFS may require the same circulating EBV-encoded proteins. Our recent findings of the prolonged presence of elevated serum antibodies to the encoded proteins EBV dUTPase and EBV DNA polymerase in the EBV subset of CFS, are consistent with this paradigm. This unifying concept for the IM and EBV subsets of CFS requires confirmation with a larger group of CFS patients and with appropriate controls. There is no in vitro model for EBV lytic replication, but the murine gammaherpesvirus 68 establishes a latent infection in mouse B-lymphocytes similar to that of EBV.[62,63] We are aware that we offer a previously unrecognized herpesvirus pathogenesis for CFS and IM. The presence of unassembled circulating EBV early proteins such as the enzymes EBV DNA polymerase and EBV dUTPase may facilitate a science-based CFS diagnosis, and provide proof for the need for long-term antiviral therapy (Table 1).

Table 1 Paradigm: EBV replication in healthy immune people, and the EBV subset of CFS patients

EBV	Healthy EBV immune people	CFS EBV subset patients
Virions (saliva)	+	+
Memory β-cell (periphery)	+	+
VCA, IgM (serum)[a]	–	–[a]
VCA, IgG (serum)	+	+
EA(D) (serum)[b]	–[b]	+
Antibody to DNA polymerase (serum)	–	+
Antibody to dUTPase (serum)	–	+

Notes: [a]EBV VCA IgM serum antibody may be present in 10%–15% of CFS patients.[55] [b]EBV EA(D) serum antibody may be in approximately 30% of healthy immune people.

Abbreviations: CFS, chronic fatigue syndrome; EA(D), early antigen (diffuse); EBV, Epstein–Barr virus; VCA, viral capsid antigen.

Disclosure

The authors report the following conflicts: A Martin Lerner and Safedin Beqaj have ownership of CFS LLC, which owns US patents for diagnosis and treatment of CFS and pending patents distinguishing groups A and B CFS:

Patent number	Patent title
6,894,056	Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome
6,537,997	Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome
6,399,622	Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome
6,258,818	Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome
5,872,123	Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome
5,464,020	Diagnosing and treating subacute cardiac dysfunction
5,357,968	Diagnosing and treating subacute myocarditis
5,213,106	Diagnosing and treating chronic fatigue syndrome by electrocardiographic monitoring of T-waves
Pending	Methods for diagnosis and treatment of chronic fatigue syndrome

Ohio State University and CFS LLC have submitted a patent, "EBV DNA polymerase and EBV dUTPase in the EBV subset of CFS." Additionally, CFS LLC owns Certificates of Registration issued under the seal of the Copyright Office for the following works: Functional Activity Appraisal Energy Index Score; Health Care Worker Assessment; Quantitative CFS Physical Activity Assessment; Grading Scale for Energy Index Questionnaire; CFS: Based Upon Functional Capacity; EIPS: Energy Index Point Score Chart; A Functional Capacity Measurement Tool for Chronic Fatigue Syndrome (CFS) Patients. Safedin Beqaj is employed by Pathology, Inc. There are no further patents, products in development, marketed products, or other conflicts of interest to declare.

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