Epstein-Barr Virus: In Search of a Causal or a Casual Relationship Between the Virus and the Disease?

Efthymios Dardiotis MD PhD[1] and Dimitrios P. Bogdanos MD PhD[2]

[1]Departments of Neurology and [2]Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

KEY WORDS: autoantibody, autoimmunity, molecular mimicry, Epstein–Barr virus (EBV)

IMAJ 2018; 20: 111–113

Epstein–Barr virus (EBV) is a human herpes virus that infects more than 90% of individuals. EBV causes persistent latent infection with sporadic reactivations. The virus is also able to transform B lymphocytes, and at times to induce strong T-cell responses. These characteristics make the virus a likely trigger of immune-mediated diseases and a plausible cause of autoimmunity, and indeed a full-blown autoimmune disease. At the other end of the spectrum, EBV can be a cause of lymphoma or other viral-mediated inflammatory disorders, which are caused by the close interplay between the host's immune system and the encounter with the virus.

Hence, it comes as no surprise that the virus is aetiologically linked with a plethora of heterogenous organ or non-organ specific diseases. Hypotheses driven scenarios, supported by epidemiological, immunological, and virological data in human diseases, have strengthened the perceived notion that EBV stands as one of the strongest candidates for the induction of autoimmune diseases through various mechanisms, the most frequently incriminated being molecular mimicry among immunodominant EBV antigens and autoantigenic epitopes.

Despite their limitations, Koch's postulates are still a useful tool in judging whether a pathogen's impact can be interpreted as causation with respect to some target disease. According to Koch's four postulates, the pathogen must be present in all cases of the disease, the microorganism must/can be isolated from the affected host and grown in pure culture, the pathogen from the pure culture must cause the disease when inoculated into a healthy and susceptible laboratory animal, and the pathogen must be re-isolated from the new host and shown to be the same as the originally inoculated pathogen.

Among the reported studies, most data support a link based on the fact that immune responses against EBV (humoral or cellular) are more frequently found in those with autoimmune diseases than in healthy individuals, even when normal controls are matched for age and gender. Researchers opposing the pathogenic connection of EBV with autoimmunity claim that alterations in the frequency of antibodies (or cellular immune responses) against EBV can be the consequence rather than the foundation of disease initiation. If correct, affected individuals are more prone to develop EBV infection, which may explain the findings of increased prevalence of EBV exposure in patients with autoimmune diseases or immune mediated diseases (with no apparent autoimmune origin), in general.

Most studies [1-7] have tested antibody responses in patients with a given autoimmune disease compared to healthy controls. If anti-EBV antibody responses are found to be more frequent in affected individuals, an apparent causal relationship warrants further investigation. These tests are mainly based on antibody testing using an antigenic source with whole viral extracts or the major immunodominant antigens of EBV. The antigens usually include EBV viral capsid antigen (VCA) and Epstein-Barr nuclear antigens (EBNA). Data analyses may also include comparisons of antibody concentrations among patients and healthy individuals. Some studies do not report differences on the prevalence of anti-EBV antibodies but can find more elevated antibody titers against EBV in patients than in healthy controls. The frequency of the magnitude of anti-EBV antibodies may differ if the analysis involves reactivities against the disease under investigation and pathological cohorts including other autoimmune or non-autoimmune diseases [1-3,5,8,9]. Those reports further strengthened the aetiological association between the virus and the disease, as they report a disease-specific finding. Well-designed studies also take into consideration in their analyses cohort numbers and origin [3], demographic parameters [10,11], methodological aspects [2,3], selection and source of cases and controls [2,3], technical issues of proper antibody testing [2,3], and ideal cut-off points based on proper receiver operating characteristic (ROC) curve analysis [8,12]. Their results are considered more scientifically-sound and relevant.

A series of studies [6,12-15] attempted to identify a direct causal relationship between the virus and the disease. The hypothesis was that if the virus is responsible for the disease, polymerase chain reaction products of the isolated virus could (must) be found in samples from the affected tissue (i.e., salivary glands, brain, kidney, liver) and that these could be more prevalent in patients with the disease compared to those who are disease free, if their tissue samples are also tested [15]. While some studies are able to show evidence of viral products in tissues, the great majority are unable to do so [8,12]. This

finding by no means implies that the virus (the immunogen) needs to replicate in the damaged tissue at the time of diagnosis as an immune-mediated cell injury can occur after the immunogen has been removed in a "hit-and-run" immunological scenario. Hence, it is almost impossible for disease development and progression to depict an ontologic delineation within the indefinite stream of procedures between causal and non-causal (casual) associations involving potential triggers.

If there is no compelling evidence against the ability of EBV to potentially cause the disease, it may originate from sufficient evidence through epidemiologic studies. Such studies shed light on this issue from another angle. A demonstration of causality, especially for EBV, would require evidence in which a reduction in the frequency or severity of EBV infection would be followed by a reduction in the frequency of the autoimmune disease [16]. In other words, an indirect proof would require epidemiologic evidence that prevention of EBV infection is accompanied by a decline in disease incidence. EBV lacks a proper vaccine. Thus, EBV does not belong to the class of viruses whereby vaccination policies and prevention have given us the opportunity to witness a sharp decrease of virus-related immune mediated disorders or EBV-related lymphomas. It is also important to note that for those viruses with available vaccines, there is always the possibility that we may encounter rare cases of a temporal link between the vaccine and the disease due to unwanted adverse reactions caused by the adjuvant, a state known as autoimmune/inflammatory syndrome induced by adjuvants (ASIA), or because of harmful cross-reactive immune responses due to molecular mimicry between the virus and autoantigenic epitopes [17]. Poor evidence of causality provided for EBV originates from data on experimental models of autoimmune diseases, such as those reported on experimental autoimmune encephalomyelitis (EAE) and the animal models of multiple sclerosis.The limited data are mainly due to the fact that EBV only infects humans and studies are based on γ-herpesvirus-68 (γHV-68), a murine γ-herpesvirus that is used as a model to investigate EBV and Kaposi's sarcoma-associated herpes virus human γ-herpesviruses. γHV-68 shares most of its genomes with these two viruses. Equally important is that some γHV-68 genes are associated with EBV cell tropism and latency. Transformation γHV-68 infected mice show exacerbated symptoms of EAE compared to non-infected mice.

However, there are diseases showing not only a strong association with the virus but also an arguably pathogenic link showing that the virus is responsible for disease induction. The connection of EBV with EBV-related lymphoma has been known for many years. The aetiological connection between EBV and hemophagocytic lymphohistiocytosis (HLH) is also well described [18]. In this issue of *IMAJ*, Shao and co authors [19] performed a retrospective single-institution study investigating 23 adult patients with EBV-associated HLH during a 15 year period. EBV-HLH is a major subtype of secondary HLH that is developed due to primary EBV infection. Other less frequent causes of virus associated HLV include cytomegalovirus, varicella zoster virus, herpes simplex virus, parvovirus, and human immunodeficiency virus. EBV affects all ages, from infants to young adults, and appears mostly in immunocompetent individuals. Although associated with substantial morbidity and mortality, early diagnosis and prompt treatment may result in successful management of the disorder.

The need to understand the epidemiology of the disease has prompted investigators to review the clinical characteristics of the largest series from Asian patients over the past decade. The awareness of the disease has increased over the last few years and molecular methods to trace the virus also recently became available and more easily accessible in routine laboratory tests. Thus, it is not surprising that all EBV-HLH cases were reported after 2002, when EBV-HLH testing became more common. Several findings are of interest and may help us to better understand the disease. In terms of the geo-epidemiology, Shao et al. [19] reported that rural residents may be more prone to EBV-HLH than residents living in urban locations, with no further explanation. A slight predominance of male gender was also found. Most patients were < 30 years.

Laboratory testing must include EBV DNA viral load, as it appears to be (as was expected) more helpful in comparing serological tests of the virus. Other common laboratory findings included significant typical hyperferritinemia, with > 10000 μg/L in two-thirds of the patients, elevated LDH, transaminasaemia, thrombocytopenia, coagulopathy, and hypofibrinogenemia. Flow cytometric measurements of lymphocyte subsets assisted the diagnostic testing as a significant decline of $CD4^+$/$CD8^+$ T and NK cells was present in 50% of the patients. Patients also experienced high fever and signs of splenomegaly.

The aetiology of EBV remains poorly understood. The most perplexing issue, however, concerns efficient treatment and accurate estimation of prognosis.

The poor prognostic factors of EBV-HLH include older age, low platelet count, high AST and elevated LDH levels, and underlying lymphoma. Thus, searching for lymphomas is a high priority for the management of the patients. Poor performance and bone marrow suppression are also bad prognostic indicators. Currently, the ideal therapy for patients with EBV-HLH remains unknown. Although HLH-2004 protocol has raised expectations, better treatments are urgently needed for the adult population.

A study by Beader and colleagues [20], which is included in this issue of *IMAJ*, assessed serological evidence of EBV infection in various cohorts in 2022 consecutive sera in a 2 year period. The study from Croatia tested immunoglobulin M (IgM) and IgG antibodies against VCA of EBV, not only in the general population but also in patient cohorts consisted of patients with multiple sclerosis and Crohn's disease, hemodialysis patients, heart transplant recipients, and those with hematological malignancies. Despite the selection of heterogeneity not immediately apparent in

diseases tested for antibodies against EBV, the findings of the study are of interest, especially since the authors tested IgG avidity to determine evidence of recent primary infection in various age groups.

EBV is widespread in the Croatian population, a finding that was expected. Older age appeared to be the main risk factor for EBV seropositivity, a finding that is in agreement with what we know for the seroprevalence of the virus. Approximately 60% of Croatian children were infected with EBV by the age of 9 years and 79.0% by 19 years. Again, these results are within the range of published data from other countries. The bimodal pattern shown in some studies was not confirmed.

Many observations implicate EBV in the pathogenesis of multiple sclerosis [9]. The evidence linking EBV infection to multiple sclerosis comes from epidemiological studies showing that nearly all adult patients with multiple sclerosis are seropositive for EBV. In the study by Beader and co-authors, practically all Croatian children (98.6%) with multiple sclerosis had detectable anti-EBV VCA antibodies (the highest among cohorts) compared to just 72.1% of controls; but the numbers are small to allow extensive discussion concerning the potential causal relation between the virus and this autoimmune disease.

There is always the possibility that the infection is epiphenomenal and comes as a consequence of the immunosuppressed state of these children due to treatment regimens. Of interest, patients affected with Crohn's disease, another autoimmune disease, showed similar frequency of EBV seroprevalence to the general population, making it impossible for the authors to raise pathogenic connotations.

There is no doubt that we will learn more from the undisputed connection between EBV and HLH. This peculiar bond caused by a cytokine storm initiated by EBV-induced cell subset activation, is a good model to study the perplexed nature of virus/host intercations. We are also in search of a definite answer as to whether EBV is a decisive marker of autoimmune diseases, including multiple sclerosis. This remains a puzzling issue and will be difficult to solve in the future. Nevertheless, studies as those published in this issue of *IMAJ* improve our efforts to understand the close interplay between EBV and host and their casual or causal relationship.

Correspondence
Dr. D.P. Bogdanos
Dept. of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, Larissa 40500, Greece
Phone: (30-241) 350-2766
Fax: (30-241) 350-1016
email: bogdanos@med.uth.gr

References

1. Inoue N, Harada S, Miyasaka N, Oya A, Yanagi K. Analysis of antibody titers to Epstein-Barr virus nuclear antigens in sera of patients with Sjogren's syndrome and with rheumatoid arthritis. *J Infect Dis* 1991; *164:* 22-8.
2. Almohmeed YH, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PLoS One* 2013; 8: e61110.
3. Ball RJ, Avenell A, Aucott L, Hanlon P, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein-Barr virus and rheumatoid arthritis. *Arthritis Res Ther* 2015; 17: 274.
4. Hanlon P, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein-Barr virus and systemic lupus erythematosus. *Arthritis Res Ther* 2014; 16: R3.
5. Kivity S, Arango MT, Ehrenfeld M, et al. Infection and autoimmunity in Sjogren's syndrome: a clinical study and comprehensive review. *J Autoimmun* 2014; 51:17-22.
6. Rigopoulou EI, Smyk DS, Matthews CE, et al. Epstein-Barr virus as a trigger of autoimmune liver diseases. *Adv Virol* 2012; 2012: 987471.
7. Sakkas LI, Daoussis D, Liossis SN, Bogdanos DP. The infectious basis of ACPA-positive rheumatoid arthritis. *Front Microbiol* 2017; 8: 1853.
8. Draborg AH, Duus K, Houen G. Epstein-Barr virus in systemic autoimmune diseases. *Clin Dev Immunol* 2013; 2013: 535738.
9. Cuomo L, Cirone M, Di Gregorio AO, et al. Elevated antinuclear antibodies and altered anti-Epstein-Barr virus immune responses. *Virus Res* 2015; 195: 95-9.
10. Disanto G, Pakpoor J, Morahan JM, et al. Epstein-Barr virus, latitude and multiple sclerosis. *Mult Scler* 2013; 19: 362-5.
11. Grant WB. Latitude and multiple sclerosis prevalence: vitamin D reduces risk of Epstein-Barr virus infection. *Mult Scler* 2010, 16: 373; author reply 374-5.
12. Draborg A, Izarzugaza JM, Houen G. How compelling are the data for Epstein-Barr virus being a trigger for systemic lupus and other autoimmune diseases? *Curr Opin Rheumatol* 2016; 28: 398-404.
13. Dreyfus DH. Role of T cells in EBV-infected systemic lupus erythematosus patients. *J Immunol* 2005, 175: 3460; author reply 3461.
14. Gross AJ, Hochberg D, Rand WM, Thorley-Lawson DA. EBV and systemic lupus erythematosus: a new perspective. *J Immuno* 2005; 174: 6599-607.
15. Blaschke S, Schwarz G, Moneke D, et al. Epstein-Barr virus infection in peripheral blood mononuclear cells, synovial fluid cells, and synovial membranes of patients with rheumatoid arthritis. *J Rheumatol* 2000; 27: 866-73.
16. Willett W, Greenland S, MacMahon B, et al. The discipline of epidemiology. *Science* 1995; 269: 1325-6.
17. Watad A, Quaresma M, Bragazzi NL, et al. The autoimmune/inflammatory syndrome induced by adjuvants (ASIA)/Shoenfeld's syndrome: descriptive analysis of 300 patients from the international ASIA syndrome registry. *Clin Rheumatol* 2017; doi: 10.1007/s10067-017-3748-9. [Epub ahead of print]
18. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. *Blood* 2011; 118: 4041-52.
19. Shao X, Xu Y, Xu X, Xu Y, Chen H, Hong M, Liu L. Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in adults: a retrospective analysis of 23 patients in China and review of the literature. *IMAJ* 2018; 20 (2): 80-85.
20. Beader N, Kolaric B, Slacanac D, Tabain I, Vilibic-Cavlek T. Seroepidemiological study of Epstein-Barr virus in different population groups in Croatia. *IMAJ* 2018; 20 (2): 86-90.

"The tools of conquest do not necessarily come with bombs, and explosions, and fallout. There are weapons that are simply thoughts, attitudes, prejudices, to be found only in the minds of men. For the record, prejudices can kill and suspicion can destroy; and a thoughtless, frightened search for a scapegoat has a fallout all of its own for the children, and the children yet unborn"

Rod Serling, (1924–1975), writer of the science fiction TV series *The Twilight Zone*