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Commentary OMICS International

A Commentary on the Use of Epstein-Barr Virus Specific Antibodies as Biological Markers in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS), a chronic demyelinating and neurodegenerative disease of the central nervous system (CNS), whose pathogenesis likely involves an interaction of environmental factors with a genetic predisposition. The hypothesis that Epstein-Barr virus (EBV), a ubiquitous human γ-herpesvirus may be a causal agent pivots on the evidence of EBV-specific antibodies high titers in MS patients as compared to controls, and on the observed direct association between such antibodies titers and disease activity. However, the literature on the possible etiological role of EBV is conflicting. This commentary aims to provide an overview on the use of EBV-specific antibodies as biomarkers in MS course.

Keywords: Multiple sclerosis; Epstein-Barr virus; Antibodies; Biomarkers

Commentary

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative immune-mediated disease of the central nervous system (CNS) and the commonest cause of non-traumatic neurological disability in young adults [1]. Among the main diagnostic features of MS are: a) clinical spatial and temporal dissemination of neurological sign and symptoms; b) multi-focal lesions in the periventricular white matter on Magnetic Resonance Imaging (MRI) scans [2]; c) the presence of oligoclonal IgG bands (OCB) in cerebrospinal fluid (CSF) and not in serum, reflecting an intrathecal synthesis of immunoglobulins [3].

Despite MS etiopathogenesis remains largely unknown, it is considered a multifaceted demyelinating and neurodegenerative disorder likely generated by an age-specific interplay between genetic predisposition and environmental factors [1]. The potential role for an infectious agent in MS pathogenesis has been supported by epidemiological evidences [4,5].

Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus capable to infect, activate, and latently persist in B lymphocytes for the lifetime of the infected host [6]. Primary infection with EBV is transmitted through saliva and it is asymptomatic, if occurring in childhood, or can cause infectious mononucleosis (IM) in puberty or adulthood [7].

An increasing number of articles have been published in the last decades on the association between MS and EBV (Figure 1) and special interest was raised by Serafini and colleagues who demonstrated EBV-infected infiltrating B lymphocytes in post-mortem brain tissue of MS patients [8]. However, other groups failed to consistently find EBV-positive B cells in MS affected brains [9].

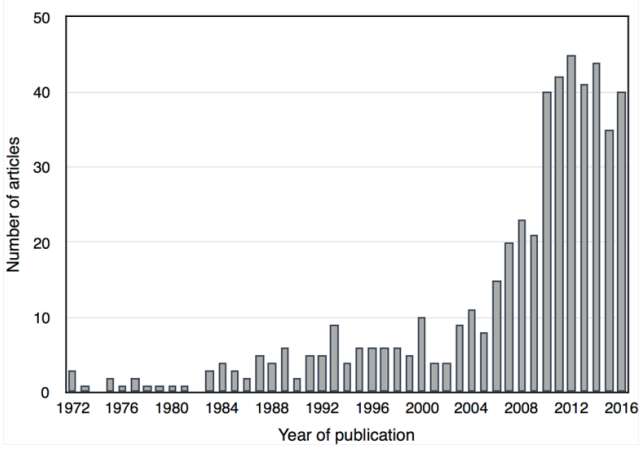

Figure 1: Number of articles published per year matching the searching terms "multiple sclerosis" and "EBV" on PubMed.

The strongest association between MS and EBV still derives from seroepidemiological investigations (Table 1) suggesting the use of EBV-specific antibodies as markers of the natural course of the disease through the longitudinal correlation with known clinical variables (type 0 biomarkers) [10]. Most such evidences build from the use of the Epstein-Barr nuclear antigen (EBNA) complex, especially EBNA-1 and the structural protein viral capsid antigen (VCA), as targets of the humoral response. EBNA-1 is the only EBV-encoded protein expressed in proliferating EBV-infected memory B cells and it maintains EBV infection by distributing viral DNA into progeny cells during cell division [11]. VCA is expressed during acute infection or following occasional reactivations of the lytic cycle [12]. Elevated EBV-specific antibodies titers have been reported more commonly in MS patients than in controls, preceding and predicting the development of the

disease and of its progression, and were intrathecally produced in MS patients [8,13-25].

Biomarker	Correlation (Target antigens)
EBV-specific antibodies in serum	More elevated in MS patients than in controls (EBNA-1) [13]
	Elevated in serum of pediatric MS patients (EBNA and VCA) [14,15]
	More elevated before the onset of the disease (EBNA-2) [16]
	Robust marker of MS risk (EBNA) [17]
	Increased in CIS patients; predicted conversion to MS; correlated to disease progression (EBNA-1) [18, 19]
	Associated to grey matter atrophy (VCA) [20] and to cortical atrophy and lesion burden (EBNA-1 and VCA) [21]
EBV-specific antibodies in CSF	Intrethecally synthesized (AI positive) more in MS than in controls (EBNA-1 and VCA) [22]
	CSF EBV-specific OCB in 30% of MS patients (EBNA-1) [23]
	CSF EBV-specific OCB in 94% [8], 24% [24] and 14% [25] of MS patients (all viral antigens)

Table 1: Epstein-Barr virus (EBV) specific antibodies as biological markers to sustain a causal role for EBV in multiple sclerosis (MS) pathogenesis (AI: Antibody Index; CIS: Clinically Isolated Syndrome; CSF: Cerebrospinal Fluid; EBNA: Epstein-Barr Nuclear Antigen; EBV: Epstein-Barr Virus; MS: Multiple Sclerosis; OCB: Oligoclonal IgG Bands; VCA: Viral Capsid Antigen).

Notwithstanding a general agreement on the association between MS and elevated serum concentrations of anti-EBV (especially anti-EBNA-1 specific) antibodies, other evidences argue against the use of such antibodies as biomarkers in MS (Table 2). In particular, serum levels of EBV-specific antibodies were not found to correlate with disease severity, progression and activity by us [24] and by Ingram et al. [26] and Gieß et al. [27]. Furthermore, EBV-specific antibodies concentrations were not shown to be influenced by disease modifying treatments [28,29], apart from their use in capturing therapeutic intervention effects implying their mechanism of action (type 1 biomarkers) [10]. Also CSF anti-EBV antibody concentrations did not correlate with clinical activity, severity and duration and were found higher in controls than in MS [24,30,31]. Moreover, whereas intrathecally synthesized, EBV-specific antibodies were infrequent and with low affinity in MS [24,32]. Of particular interest is indeed the role of the antibody affinity in the context of MS. High affinity antibodies specific for the causative agent have been shown in infectious diseases [33]. Interestingly, the presence of somatic hyper mutation in the immunoglobulin genes indicates that OCB are composed of high-affinity antibodies [34]. In a recent work, we found that 24% of MS patients had EBV-specific IgG OCB in CSF and not in the corresponding serum, with a great inter-individual variability in number and intensity [24]. However, all these OCB showed low affinity for viral proteins, suggesting that they may include cross-reactive antibodies which are part of a polyspecific intrathecal response where EBV would not represent the cognate antigen. In the same study, we found that the antibody affinity was higher for the virus surface structural virocapsidic antigen, than for the nuclear antigen, released by dying cells, confirming the EBV intermittent cytopathic behaviour [24,35].

Biomarker	Correlation (Target antigens)
EBV-specific antibodies in serum	No differences between MS subgroups (RR, PP, CARR, CSRR), no correlation with age at onset, disease duration, EDSS or MSSS (EBNA-1) [26]
	No correlation with number of MRI lesions, Barkhof criteria, EDSS, and not association with conversion to clinically definite MS in CIS/early RR MS (EBNA-1 and VCA) [27]
	No correlation with disease activity (clinical and MRI) and disease duration (EBNA-1 and VCA) [24]
	Not useful for monitoring Natalizumab and interferon-beta therapy (EBNA-1 and VCA) [28,29]
EBV-specific antibodies in CSF	No correlation with disease activity (clinical and MRI) disease severity (EDSS) and disease duration (EBNA-1 and VCA) [24]
	More elevated in OIND than in MS and NIND (VCA) [30]
	Intrathecal synthesis (AI) almost absent in MS, OIND and NIND (EBNA-1 and VCA) [24,31]
	EBV-specific OCB in MS, OIND and NIND as a 'mirror pattern' (all viral antigens) [32]
	EBV-specific OCB composed by low affinity antibodies (all viral antigens) [24]

Table 2: Epstein-Barr virus (EBV) specific antibodies as biological markers to argue against a causal role for EBV in multiple sclerosis (MS) pathogenesis (AI: Antibody Index; CARR: Clinical Active Relapsing Remitting Multiple Sclerosis; CIS: Clinically Isolated Syndrome; CSF: Cerebrospinal Fluid; CSRR: Clinical Stable Relapsing Remitting Multiple Sclerosis; EBNA: Epstein-Barr Nuclear Antigen; EBV: Epstein-Barr Virus; EDSS: Expanded Disability Status Scale; MRI: Magnetic Resonance Imaging; MS: Multiple Sclerosis; MSSS: Multiple Sclerosis Severity Score; OCB: Oligoclonal IgG Bands; RR: Relapsing Remitting Multiple Sclerosis; PP: Primary Progressive Multiple Sclerosis; VCA: Viral Capsid Antigen).

EBV is widespread in the human population, often nearly or completely asymptomatic. It persists life-long in B cells and intermittently causes lytic infections. For these reasons the association between MS and EBV do not fulfil the Koch's Postulates for causality, yielding discordant results which per se fail to clarify the nature of this virus-specific humoral immune response.

New hypotheses which could explain the association between EBV and MS have been proposed [36] and EBV has been indicated as the possible trigger of an intrathecal reaction that occurs during MS [37]. However, the conceptual frame for EBV-related pathogenetic mechanism in MS builds on the role of EBV-transformed B lymphocytes infiltrating the brain, maintaining the intrathecal production of antibodies [38] and/or acting as resident antigen presenting cells (APC) sustaining the immune-mediated reaction within the CNS [39] (Figure 2). This condition may be worsened by the proliferation of latently infected cells due to the defective CD8+ T-cell control of EBV reactivation in MS patients [40], resulting in the maintenance of the autoreactive/pathogenic EBV-infected B cells reservoir for a lifetime. One indirect evidence of such hypothesis is that the antibody production in MS CSF is stable overtime [41] and that

their specificity seems to be unrelated to the disease activity [42] as consequence of a random EBV-driven B cells transformation [43].

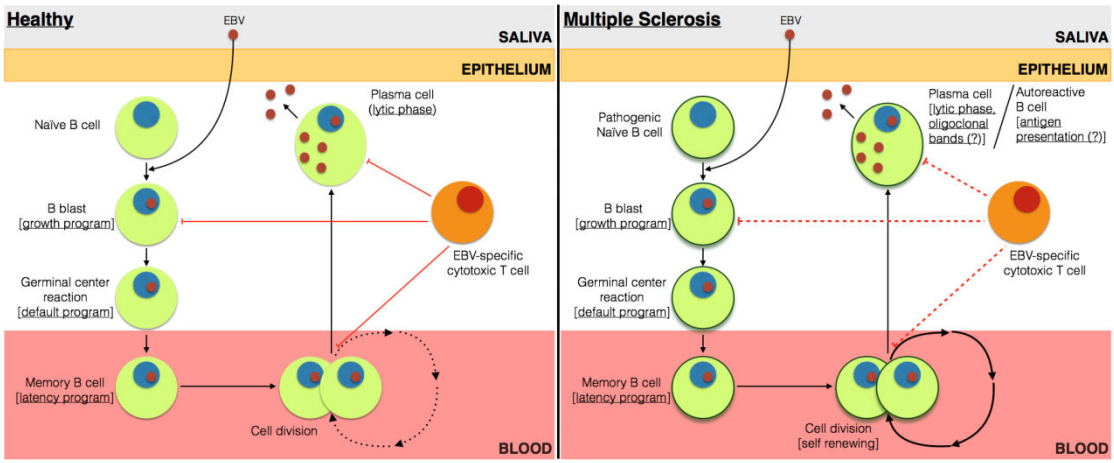

Figure 2: Epstein-Barr virus (EBV) has the ability to infect, activate and latently persist in B lymphocytes through the expression of different transcription programs. In healthy individuals (left panel), during primary infection, the virus enters the tonsil from the saliva and infects naïve B cells. EBV, expressing the "growth program" or latent program III, drives out to the resting state naïve B cells to become activated B blasts which then enter the germinal centers reaction. In this condition EBV express the "default program" or latent program II that stimulate B blasts to proliferate and differentiate into infected memory B cells that can then recirculate in blood and in which the virus does not express viral protein (latency) or expresses only the nuclear antigen 1 during cell division. Circulating memory B cells can finally differentiate into plasma cells which initiates the lytic phase of infection producing free virions. In normal condition, EBV infection is controlled by EBV-specific cytotoxic (CD8+) T lymphocytes. During multiple sclerosis (right panel) all these processes could involve pathogenetic/autoreactive naïve B cells. A repertoire of EBV-infected memory B cells could survive in the MS infected host due to an altered CD8+ T cells response to EBV [40]. Finally, CNS-infiltrating pathogenic plasma cells could contribute to the development and to the maintenance of the neuroinflammation through the production of oligoclonal IgG bands [9] or acting as antigen presenting cells that stimulate autoreactive CD4+ cells [10]. Red lines and red dashed lines with perpendicular bars indicate normal and defective CD8+ T-cell control respectively. This picture is mainly based on works published by Thorley-Lawson et al. [6], for the biology of the EBV infection and by Serafini et al. for the dysregulated EBV infection [8].

In conclusion, EBV remains one of the most important environmental risk factor for MS with a potential triggering mechanism in the intrathecal IgG synthesis - MS laboratory hallmark - and this is still matter of interest. Further research will elucidate the role of a persistent dysregulated EBV infection and/or of an altered immune response to EBV in triggering or modulating the risk for MS.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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References

1. Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23: 683-747.
2. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, et al. (2011) Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 69: 292-302.
3. Gastaldi M, Zardini E, Franciotta D (2017) An update on the use of cerebrospinal fluid analysis as a diagnostic tool in multiple sclerosis. Expert Rev Mol Diagn 17: 31-46.
4. Correale J, Gaitán MI (2015) Multiple sclerosis and environmental factors: The role of vitamin D, parasites and Epstein-Barr virus infection. Acta Neurol Scand 132: 46-55.
5. Santiago O, Gutierrez J, Sorlozano A, de Dios Luna J, Villegas E, et al. (2010) Relation between Epstein-Barr virus and multiple sclerosis: analytic study of scientific production. Eur J Clin Microbiol Infect Dis 29: 857-866.
6. Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M (2013) The pathogenesis of Epstein-Barr virus persistent infection. Curr Opin Virol 3: 227-232.
7. Ascherio A, Munger KL, Lünemann JD (2012) The initiation and prevention of multiple sclerosis. Nat Rev Neurol 8: 602-612.
8. Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, et al. (2007) Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med 204: 2899-2912.
9. Lassmann H, Niedobitek G, Aloisi F, Middeldorp JM (2011) Epstein–Barr virus in the multiple sclerosis brain: a controversial issue - report on a

focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. Brain 134: 2772–2786.

10. Bielekova B, Martin R (2004) Development of biomarkers in multiple sclerosis. Brain 127: 1463-1478.
11. Münz C (2004) Epstein-barr virus nuclear antigen 1: From immunologically invisible to a promising T cell target. J Exp Med 199: 1301-1304.
12. Hislop AD, Taylor GS, Sauce D, Rickinson AB (2007) Cellular responses to viral infection in humans: Lessons from Epstein-Barr virus. Annu Rev Immunol 25: 587-617.
13. DeLorenze GN, Munger KL, Lennette ET, Orentreich N, Vogelman JH, et al. (2006) Epstein-Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. Arch Neurol 63: 839-844.
14. Pohl D, Krone B, Rostasy K, Kahler E, Brunner E, et al. (2006) High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. Neurology 67: 2063-2065.
15. Banwell B, Krupp L, Kennedy J, Tellier R, Tenembaum S, et al. (2007) Clinical features and viral serologies in children with multiple sclerosis: A multinational observational study. Lancet Neurol 6: 773-781.
16. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernán MA, et al. (2001) Epstein-Barr virus antibodies and risk of multiple sclerosis: A prospective study. JAMA 286: 3083-3088.
17. Munger KL, Levin LI, O'Reilly EJ, Falk KI, Ascherio A (2011) Anti-Epstein-Barr virus antibodies as serological markers of multiple sclerosis: a prospective study among United States military personnel. Mult Scler 17: 1185-1193.
18. Lünemann JD, Tintoré M, Messmer B, Strowig T, Rovira A, et al. (2010) Elevated Epstein-Barr virus-encoded nuclear antigen-1 immune responses predict conversion to multiple sclerosis. Ann Neurol 67: 159-169.
19. Levin LI, Munger KL, Rubertone MV, Peck CA, Lennette ET, et al. (2005) Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 293: 2496-2500.
20. Zivadinov R, Zorzon M, Weinstock-Guttman B, Serafin M, Bosco A, et al. (2009) Epstein-Barr virus is associated with grey matter atrophy in multiple sclerosis. J Neurol Neurosurg Psychiatry 80: 620-625.
21. Zivadinov R, Cerza N, Hagemeier J, Carl E, Badgett D, et al. (2016) Humoral response to EBV is associated with cortical atrophy and lesion burden in patients with MS. Neurol Neuroimmunol Neuroinflamm 3:e190.
22. Jaquiéry E, Jilek S, Schluep M, Meylan P, Lysandropoulos A, et al. (2010) Intrathecal immune responses to EBV in early MS. Eur J Immunol 40: 878-887.
23. Rand KH, Houck H, Denslow ND, Heilman KM (2000) Epstein-Barr virus nuclear antigen-1 (EBNA-1) associated oligoclonal bands in patients with multiple sclerosis. J Neurol Sci 173: 32-39.
24. Castellazzi M, Contini C, Tamborino C, Fasolo F, Roversi G, et al. (2014) Epstein-Barr virus-specific intrathecal oligoclonal IgG production in relapsing-remitting multiple sclerosis is limited to a subset of patients and is composed of low-affinity antibodies. J Neuroinflammation 11: 188.
25. Virtanen JO, Wohler J, Fenton K, Reich DS, Jacobson S (2014) Oligoclonal bands in multiple sclerosis reactive against two herpes viruses and association with magnetic resonance imaging findings. Mult Scler 20: 27-34.
26. Ingram G, Bugert JJ, Loveless S, Robertson NP (2010) Anti-EBNA-1 IgG is not a reliable marker of multiple sclerosis clinical disease activity. Eur J Neurol 17: 1386-1389.
27. Gieß RM, Pfuhl C, Behrens JR, Rasche L, Freitag E, et al. (2017) Epstein-Barr virus antibodies in serum and DNA load in saliva are not associated with radiological or clinical disease activity in patients with early multiple sclerosis. PLoS ONE 12: e0175279.
28. Raffel J, Dobson R, Gafson A, Mattoscio M, Muraro P, et al. (2014) Multiple sclerosis therapy and Epstein-Barr virus antibody titres. Mult Scler Relat Disord 3: 372-374.
29. Castellazzi M, Delbue S, Elia F, Gastaldi M, Franciotta D, et al. (2015) Epstein-Barr virus specific antibody response in multiple sclerosis patients during 21 months of Natalizumab treatment. Dis Markers 2015: 901312.
30. Castellazzi M, Tamborino C, Cani A, Negri E, Baldi E, et al. (2010) Epstein-Barr virus-specific antibody response in cerebrospinal fluid and serum of patients with multiple sclerosis. Mult Scler 16: 883-887.
31. Nociti V, Frisullo G, Marti A, Luigetti M, Iorio R, et al. (2010) Epstein-Barr virus antibodies in serum and cerebrospinal fluid from multiple sclerosis, chronic inflammatory demyelinating polyradiculoneuropathy and amyotrophic lateral sclerosis. J Neuroimmunol 225: 149-152.
32. Franciotta D, Di Stefano AL, Jarius S, Zardini E, Tavazzi E, et al. (2011) Cerebrospinal BAFF and Epstein-Barr virus-specific oligoclonal bands in multiple sclerosis and other inflammatory demyelinating neurological diseases. J Neuroimmunol 230: 160-163.
33. Luxton RW, Zeman A, Holzel H, Harvey P, Wilson J, et al. (1995) Affinity of antigen-specific IgG distinguishes multiple sclerosis from encephalitis. J Neurol Sci 132: 11-19.
34. Obermeier B, Mentele R, Malotka J, Kellermann J, Kümpfel T, et al. (2008) Matching of oligoclonal immunoglobulin transcriptomes and proteomes of cerebrospinal fluid in multiple sclerosis. Nat Med 14: 688–693.
35. Hangartner L, Zinkernagel RM, Hengartner H (2006) Antiviral antibody response: The two extremes of a wide spectrum. Nat Rev Immunol 6: 231-243.
36. Owens GP, Bennett JL (2012) Trigger, pathogen or bystander: the complex nexus linking Epstein-Barr virus and multiple sclerosis. Mult Scler 18: 1204-1208.
37. Otto C, Hofmann J, Ruprecht K (2016) Antibody producing B lineage cells invade the central nervous system predominantly at the time of and triggered by acute Epstein-Barr virus infection: A hypothesis on the origin of intrathecal immunoglobulin synthesis in multiple sclerosis. Med Hypotheses 91: 109-113.
38. Fierz W (2017) Multiple sclerosis: An example of pathogenic viral interaction? Virol J 14: 42.
39. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, et al. (2008) B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 358: 676-688.
40. Pender MP, Csurhes PA, Burrows JM, Burrows SR (2017) Defective T-cell control of Epstein-Barr virus infection in multiple sclerosis. Clin Transl Immunology 6: e126.
41. Petzold A (2013) Intrathecal oligoclonal IgG synthesis in multiple sclerosis. J Neuroimmunol 262: 1-10.
42. Willis SN, Stathopoulos P, Chastre A, Compton SD, Hafler DA, et al. (2015) Investigating the antigen specificity of multiple sclerosis central nervous system-derived immunoglobulins. Front Immunol 6: 600.
43. Tosato G, Blaese RM, Yarchoan R (1985) Relationship between immunoglobulin production and immortalization by Epstein Barr virus. J Immunol 135: 959-964.

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