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Review article

EBV and MS: Major cause, minor contribution or red-herring?

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ABSTRACT

Multiple Sclerosis (MS) is a chronic neurological disease with genetic and environmental risk factors. Epstein Barr-Virus (EBV) has been closely associated with MS but with a significant amount of conflicting evidence. Some of the evidence for EBV involvement in MS includes: almost 100% of MS patients showing past EBV infection, an association with Infectious Mononucleosis (acute EBV infection), higher titres of EBV antibodies associated with an increased risk of MS development, and an overall altered immune response to EBV found in peripheral blood and the CNS of MS patients. However, evidence for EBV presence in the CSF and T cell responses to EBV in MS have been particularly conflicting. Several hypotheses have been proposed for direct and indirect EBV involvement in MS such as 1) Molecular Mimicry 2) Mistaken Self 3) Bystander Damage and 4) Autoreactive B cells infected with EBV. More recently, an association between EBV and human endogenous retrovirus in MS has been shown, which may provide an alternative pathogenetic target for MS treatment. However, if EBV is not the major contributor to MS and is instead one of several viral or infectious agents able to elicit a similar altered immune response, MS development may be the result of a failure of viral clearance in general. This review aims to evaluate the evidence for the currently discussed theories of EBV involvement in MS pathogenesis.

1. Introduction

Multiple Sclerosis (MS) is a chronic neurological disease affecting more than 23,000 individuals in Australia and approximately 2.5 million people worldwide (Hollenbach and Oksenberg, 2015). Three times as many woman as men (Ribbons et al., 2016) are now affected by MS and the incidence of MS is increasing in ethnic groups other than European Caucasians. MS is associated with neurodegeneration and central nervous system (CNS) inflammation mediated by an aberrant immune system and characterised by an altered T Cell response (Carbajal et al., 2015) to self.

While there have been significant improvements in the treatments of MS over the last decade, the underlying cause and pathogenesis of MS remains unclear. Advances in technologies such as Next Generation Sequencing and bioinformatics has enabled the identities of approximately one third of the genetic susceptibilities to MS to be localized to the Major Histocompatibility Complex (MHC), and an additional 110 polymorphisms centred on 103 discrete loci outside the MHC (Hollenbach and Oksenberg, 2015). Notwithstanding, these genetic risk factors still only account for ~30% of total disease risk. The recognised contribution of genetic risk in MS is likely to increase as technologies improve, multi-centre focused initiatives increase and genetic pathway/interaction analysis between gene-gene and gene-environmental links are better understood. Epidemiological risk factors for MS have been increasingly implicated with MS pathogenesis but require further investigation to differentiate simple association with genuine pathogenesis. The question remains, are these observations the result of an altered systemic immune system because of MS, or do they have a genuine bearing on MS pathogenesis or disease progression? The environmental risk factors most closely investigated to date, include: obesity, smoking, reduced sunlight exposure (Vitamin D deficiency) and Epstein-Barr Virus (EBV) infection (Goodin, 2016).

Out of these risk factors, EBV has been significantly implicated in MS pathogenesis. The most troubling question for EBV involvement in MS is that since the majority of the population are infected by EBV - why do some people develop MS, while others may develop cancer, and

Abbreviations: CNS, Central Nervous System; CSF, Cerebral Spinal Fluid; EBV, Epstein-Barr Virus; HC, Healthy Control; EAE, Experimental Autoimmune Encephalomyelitis; HERVS, human endogenous retroviruses; LCMV, lymphocytic choriomeningitis virus; MHC, Major Histocompatibility Complex; MOG, Myelin Oligodendrocyte Glycoprotein; MS, Multiple Sclerosis; MSRV, Multiple Sclerosis Associated Retrovirus; PBMCs, Peripheral Blood Mononuclear Cells

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most individuals are asymptomatic with no further health problems? This review evaluates the current evidence for EBV involvement in MS in relation to disease pathogenesis.

2. Epstein-Barr virus biology and route of infection

EBV is a double stranded human herpesvirus that has infected more than 90% of the population worldwide (Gao et al., 2006) and results in lifelong infection. The majority of EBV infections are asymptomatic and occur in early childhood, but if EBV is contracted later in adolescence then Infectious Mononucleosis (IM) is more likely to develop, with varying degrees of clinical severity (Dunmire et al., 2015). The prevalence of EBV infection among pre-adolescences is lower and varies significantly depending on age, geographic location and race/ethnicity (Condon et al., 2014).

EBV transmission typically occurs via sharing of infected saliva. Nearly all of those who are EBV seropositive shed virus into their saliva and are capable of infecting EBV naïve individuals. However, primary EBV infection is also possible via hematopoietic cell transplantation, solid organ transplantation and blood transfusion (Dunmire et al., 2015). EBV is capable of infecting epithelial cells, B cells as well as Natural Killer and T cells (Kang and Kieff, 2015). The primary route of infection initiates via oropharyngeal epithelium where active viral replication occurs, also known as lytic infection (Ok et al., 2015). Following this lytic cycle, EBV infects nearby naïve B cells via viral enveloping of the protruding glycoprotein on EBV GP350, with complement Receptor 2 (CD-21) on B-Cells (Ok et al., 2015; Pender and Burrows, 2014). Once EBV infects B cells it is able to turn them into active B blasts. EBV then activates its 'growth programme' (latency III), turning these B blasts into resting memory B cells (Pender and Burrows, 2014). The virus enters the latent stage after infecting naïve B cells, which is characterised by a drastic reduction in the number of proteins and miRNA expressed and can be divided into 3 stages (latency types I-III), depending on the combination of proteins expressed (Kang and Kieff, 2015). These proteins include: 6 nuclear antigens (EBNA-1, 2, 3A, 3B, 3C and LP), 3 latent membrane proteins (LMP-1, 2A and 2B) and non-coding RNA (EBER-1 and 2) (Ok et al., 2015). During lytic infection the EBV genome is linear and is capable of expressing all of its encoded proteins (approximately 100 viral proteins) and non-coding RNA, but circularises forming an episome in the nucleus of infected B cells during latent infection (Ok et al., 2015).

An alternative mechanism by which EBV persists within cells is by integrating into the host cell genome. Several studies have shown the ability of EBV to integrate within chromosomes successfully (Gao et al., 2006; Santpere et al., 2014), and indeed EBV integration of B cells *in vitro* is often used to establish immortal cell lines. However, studies into EBV DNA integration have been difficult due to methylated EBV DNA and multiple copies of viral episomes that create interference impeding mapping studies (Gao et al., 2006; Takakuwa et al., 2004). The integration of EBV has been shown to be random in cell lines (Gao et al., 2006; Santpere et al., 2014; Takakuwa et al., 2004), which may explain why some individuals infected by EBV develop EBV-related diseases compared to others. Furthermore, Hernando et. al 2013 has shown *in vitro* that EBV B cell infection causes hypomethylation, resulting in overexpression of approximately 250 genes (Hernando et al., 2013). If this occurs *in vivo,* in combination with random integration, this could further alter methylation patterns caused by EBV and potentially drive different EBV related diseases such as lymphomas and MS.

3. The association of EBV and MS

3.1. History of IM

As previously mentioned, IM is more commonly caused by late EBV infection compared to asymptomatic individuals who are infected by EBV earlier in life. There is a large amount of evidence associating IM with MS. In 2010 A meta-analysis of past history for IM and development of MS produced a relative risk of 2.17 (95% CI 1.97–2.39) (Handel et al., 2010). Sundqvist et al. later confirmed this in another meta-analysis with an odds ratio of 1.89 (1.45–2.48 95% CI) (Sundqvist et al., 2012). It is important to note that some of these studies rely on self-reported data, which is prone to error, some of which is the result of 'IM like symptoms' caused by other viruses, such as cytomegalovirus (Dunmire et al., 2015). However, Goldacre et al. showed a fourfold increase in MS risk following hospital admission with confirmed IM (Goldacre et al., 2004). The mean onset to MS following IM in this study was 14 years (Goldacre et al., 2004) compared to a large Danish cohort, which showed an increased risk of MS after 10 years and persisted even after 30 years following IM (Nielsen et al., 2007). Sundqvist et al. also showed an association between IM infection and the presence of the risk allele DRB1*15 with an attributable proportion score of 0.34 (0.001–0.68 95% CI), compared to those without a history of IM and with the presence or absence of DRB1*15 (Sundqvist et al., 2012). This further implicates the role of acute EBV infections in the development of MS.

3.2. Seropositivity in MS

Approximately 95% of the world's population is thought to have been infected by EBV at some point in their lives (Luzuriaga and Sullivan, 2010). This has been supported by 80–95% of healthy controls (HCs) consistently showing EBV seropositivity (Lucas et al., 2011). Intriguingly, nearly every study reporting EBV seropositivity in MS patients has shown > 99% are infected. A recent meta-analysis has argued that the true value for MS patients seropositive for EBV is actually 100% since those studies showing anything less, used only a single EBV detection method (Pakpoor et al., 2013). Whereas, studies that used a combination of two different EBV test methodologies confirmed 100% of MS patients as seropositive. However, this restricted the meta-analysis to only 14.6% of cases (n = 402/2760) (Pakpoor et al., 2013). In a longitudinal study of US army personnel, all individuals who were found to be seronegative for EBV seroconverted prior to MS onset, compared to only 35.7% of controls who were initially seronegative (Levin et al., 2010). This evidence has given rise to the notion that infection by EBV is a pre-requisite to the development of MS. However, an EBV serology study on paediatric MS patients using two methods of detection (ELISA and Indirect Immunofluorescence) determined 2 out of 147 (1.4%) to be seronegative. This could be the result of false negative results, but this is less likely due to the utilisation of two methodologies applied simultaneously. Furthermore, pathogenesis of paediatric MS may also be slightly different to that of adult onset disease. Therefore, the inconsistency of the results does not confirm the argument that EBV seroconversion is a prerequisite for MS. To resolve this issue further testing using a consistently accurate assay in a large patient cohort is required.

EBV titre levels were also shown to modulate disease risk in relation to other known genetic and behavioural MS risk factors such as the presence of DRB1*15, absence of A*02 and smoking. Higher Anti-EBNA-1 levels and the presence of DRB1*15 have been shown to be independent risk factors for MS but interact additively (De Jager et al., 2008). Jager et al. showed women with DRB1*15 and higher anti-EBNA-1 levels had a nine-fold increased risk of MS compared to those with lower EBNA-1 levels and DRB1*15 presence. Sundqvist et al. confirmed this in a larger cohort and went on to confirm that anti-EBNA-1: 385-420 IgG was a stronger risk marker than anti-EBNA IgG (Sundqvist et al., 2012; Sundstrom et al., 2009). Furthermore, a 16-fold increase in risk was identified, in an additive but not multiplicative interaction, between higher anti-EBNA-1: 385-420 IgG, presence of DRB1*15 and absence of A*02. Interestingly, higher levels of anti-EBNA IgG were also shown to interact additively with smoking to increase MS risk (Simon et al., 2010). The incidence of IM and smoking on MS risk was found to compete with one another, producing a negative