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Investigating the link between MS and the EBV virus

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Abstract:

Multiple sclerosis (MS) is a disease of the central nervous system characterized by inflammation, demyelination, and neuronal damage. Epstein-Barr virus (EBV) is a human DNA herpes virus infecting more than 90% of the world's population. EBV is the etiological agent of infectious mononucleosis (Pfeiffer's disease). Major predisposing factors for MS are certain tissue types (e.g., HLA DRB1*15:01), vitamin D deficiency, smoking, obesity, and infection with Epstein-Barr virus (EBV). This review summarizes current knowledge on the association between EBV and MS.

INTRODUCTION

Multiple sclerosis (MS) is a disease affecting the central nervous system (CNS), with inflammation and demyelination of nerves, eventually resulting in nerve damage and disabilities (1). Multiple sclerosis (MS) is presently regarded as a disease with multifactorial etiology, comprising genetic as well as environmental influences (2). Already more than a century ago, Pierre Marie did state that "the cause of insular (multiple) sclerosis is intimately connected with infectious diseases" (3). MS can take different courses, most often in the form of relapsing-remitting (RR) cycles of disease activity or more rarely as a primary-progressive (PP) disease. RR MS can progress over many years and may eventually develop into a secondary-progressive (SP) disease (4). Over time, the majority of relapsing-remitting MS (RRMS) patients enter a progressive disease course in which there is a gradual worsening of clinical disability with or without superimposed relapses and eventually become secondary-progressive MS (SPMS) (5). MS often leads to severe disability, although the symptoms and clinical courses are extremely diverse from malignant forms with mortality within a few years to benign forms with few symptoms and very slow progression (6). Although MS risk is associated with environmental, neuroimmune, and genetic factors, the exact causative factor for MS

is not known. The environmental risk factors most consistently linked to MS risk are infection with Epstein-Barr virus (EBV), sun exposure/vitamin D deficiency, and smoking (7). Specific environmental exposures are relevant to both triggering MS and modulating disease course. Virus infection is one crucial environmental factor. Of all viruses considered in MS pathogenesis, EBV, a highly B cell-tropic virus, is the best-studied (8).

Evidence supporting the role of Epstein-Barr virus (EBV) infection in multiple sclerosis (MS) comes from ecological studies, observational epidemiological studies, co-occurring pathologies, and experimental laboratory-based research (9). Epstein-Barr virus, the prototype of the gammaherpesviruses, is a linear, double-stranded, 184 kb DNA virus that has a primary tropism for resting B cells (10). Epstein-Barr virus (EBV) infection results in a lifelong persistence of the virus in the host's B-lymphocytes and has been associated with numerous cancers including Burkitt's lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma (11). Early age at primary EBV infection is typically asymptomatic, but primary infection during adolescence or adulthood often manifests as infectious mononucleosis, which has been associated with a two- to threefold increased risk of MS. (12).

This review summarizes current knowledge on the

association of EBV and MS including a discussion of equivocal findings.

Etiology and Epidemiology of MS

No consensus about MS etiology exists at present and theories range from idiopathic loss of self-tolerance, over molecular mimicry to chronic virus infections. Factors involved in pathogenesis broadly group into three categories: Immune factors, Environmental factors, and Genetic associations (13). Genetic factors influencing the development of MS are in particular major histocompatibility class II (MHC II) alleles, of which some increased susceptibility (e.g., human leukocyte antigen (HLA) DRB1*15:01), while others decrease susceptibility (14). Environmental factors, including latitudinal gradients in different countries, have been well-studied phenomena. Vitamin D deficiency has been considered a possible etiology for the noted predisposition of the population in higher latitudes being affected. Different infections, including Epstein Barr virus (EBV), may also play a role. There are likely complex interactions between various environmental factors with patient genetics, and understanding these pathways is an area of ongoing research (15, 16). Smoking increases the risk of MS, but some other uses of tobacco may actually reduce the risk of MS. Other environmental compound exposures have been found to affect MS susceptibility and recently, propionic acid and the composition of the intestinal microbiota have been reported to influence or be influenced by MS (17). Virus infections have for long been suspected to be involved in MS development. Most investigations have focused on EBV, which remains the most likely candidate for a causative virus, but other viruses may also play a role. Characteristic features of MS are inflammatory foci in the CNS and intra thecal synthesis of immunoglobulins (Ig), measured as an IgG index, oligo clonal bands (OCBs), or specific antibody indexes. (18).

Approximately 400,000 individuals in the United States and 2.5 million individuals worldwide have multiple sclerosis. The disease is three-fold more common in females than in males. While the age of onset is usually between 20 to 40 years, the disease can present at any age. Almost 10% of the cases present before the age of 18 (19). The prevalence and incidence of MS in Iran are reported to range from 5.3 to 89/100,000 and 7 to 148.1/100,000, respectively. At the moment, Iran is well known for its high prevalence of MS in the world, whereas 15 years ago, it was assumed based on the MS slope hypothesis that Iran could be a low-risk area for MS with an incidence of less than 5 per 100,000 people (20).

Epstein - Barr virus (EBV)

EBV is a member of the Human Herpes Virus

(HHV) family, which also includes Herpes Simplex Virus (HSV) 1 and 2, Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), HHV 6 and 7, and Kaposi Sarcoma Virus (KSV) (21). EBV was discovered in the early 1960s in lymphoma cells cultivated from tumor biopsies obtained by Burkitt in African children with jaw tumors (22). Like other herpesviruses, the EBV has a latency phase following primary infection. It infects epithelial cells, enters the circulating B lymphocyte, and persists for the life in a latent state. According to epidemiological studies, the EBV is estimated to be positive in more than 90% of the world's population (23). Primary infection usually occurs through contact with infected saliva and is asymptomatic in young children, but in up to 40% of adolescents and adults, it results in infectious mononucleosis (IM), an acute and usually self-limited lymphoproliferative disease of a few weeks duration (22, 24).

As a counter-measure to host immune responses, EBV has evolved a multitude of immune evasion mechanisms, counteracting both host cell intracellular anti-viral processes and host extracellular innate and adaptive immune responses. Cellular anti-viral pathways are many and EBV devotes a large part of its genome to the control of cellular anti-viral apoptosis mechanisms and to immune evasion (25, 26). There are two main EBV genotypes, type 1 and type 2, or A and B, respectively, distinguished by the differences in the EBNA-2 gene, since the divergence in EBNA-2 reveals only 54% homology between the two types. EBV types 1 and 2 can further be subdivided into different virus strains (27).

Following primary infection, EBV persists for the life of the host in B-lymphocytes, in which the EBV double-stranded DNA forms an episome, typically present as a single copy at a frequency of 1 to 50 per million B-lymphocytes (28). Decreased capacity for immune control of EBV may, in some cases manifest itself as a tendency to develop EBV-related diseases, including infectious mononucleosis (IM), various cancers, MS, and other relapsing-remitting autoimmune diseases (e.g., systemic autoimmune diseases) (29).

EBV and MS

In MS, much evidence indicates a role for EBV and specifically that EBV-infected B cells have entered the CNS at some point of disease development. As described above, some of the major characteristics of MS are the presence of an elevated IgG index and OCBs in the CNS, representing various B cell clones synthesizing Abs in the CNS (30). EBV appears to be involved across the clinical spectrum of MS, including early pediatric-onset MS, established relapsing-remitting (RRMS), and progressive forms (PMS), as well as in patients with both mild and severe disease courses (31). Many studies have revealed increased

amounts and increased frequencies of EBV Abs in MS, however, such studies are hampered by the nearly ubiquitous presence of EBV in adults. Moreover, the results seem to depend somewhat on the EBV Ags used and the assay methodology. Among healthy individuals infected with EBV, MS risk increases monotonically by several folds with increasing serum titers of anti-EBNA complex and anti-EBNA-1 antibodies. Results of preliminary studies suggest that the presence of EBV in plasma and antibodies to the lytic antigen BZLF1 may also predict an increased MS risk, but these associations are weaker than those observed for antibodies to EBNA-1 (32, 33). In situ hybridization and PCR studies on brain material from MS patients have in some cases indicated the presence of EBV DNA in lesions, but other studies have yielded negative results. Immuno-histochemical studies are few, but one study has demonstrated the presence of EBV Ags in post-mortem brain tissue of MS patients (34). Infectious mononucleosis is the clinical manifestation of acute EBV infection. It is more common in adolescents and adults as compared to younger children, in whom primary EBV infection is more often clinically silent. MS and infectious mononucleosis share a similar prevalence distribution, following a latitude gradient: prevalence generally rises with increasing distance to the equator, in both the southern and the northern hemispheres. Late infection with EBV, evidenced by the occurrence of infectious mononucleosis, is therefore considered a possible risk factor for MS (35).

In a prospective nested study of 62439 women, who were followed for years to determine whether elevation in serum antibodies titers to EBV capsid antigen (VCA), nuclear antigen (EBNA, EBNA-1, and EBNA-2), diffuse and restricted early D Antigen (EA-D) and early R Antigen (EA-R) precede the occurrence of MS and its symptoms. 18 cases of MS with blood collected before disease onset, were compared with their matched controls, these women had higher serum geometric mean titers (GMT) of antibodies to EBV but no cytomegalovirus (CMV) (another member of the herpes family). Elevations were significant for antibodies to EBNA-1, EBNA-2, and EA-D. The strongest association was found for antibodies to EBNA-2; a four-fold difference in titers was associated with a relative risk (RR) of MS of 3.9. Significant but generally weaker elevations in anti-EBV antibodies were also found in an analysis of 126 cases of MS with blood collected after disease onset and their matched control (36).

Genes within the human leukocyte antigen (HLA) complex have long been known to play a crucial part in the development of MS and other autoimmune diseases. Genome-wide association studies identified the HLA allele DRB1*15:01 (HLA-DR15) as the strongest genetic risk factor for MS. Interestingly,

symptomatic primary EBV infection, IM, has been found to synergize with this main genetic risk factor HLA-DR15, leading to a 7-fold increase in MS risk. The underlying mechanism of this synergistic effect is, however, largely unknown. Efforts to unravel this interaction have so far been hampered by the lack of an adequate model to study this interaction in vivo (37).

EBV specific T-cells and autoreactive B cells in MS

Aside from B-cell-related pathologies, loss of normal function in the effector T-cell population may also underlie MS disease progression. The frequency of EBNA-1 specific CD4+ memory T cells was strikingly elevated in MS patients compared to healthy EBV carriers. Furthermore, these EBNA-1 specific T cells showed increased proliferative capacity and enhanced interferon-gamma production in healthy individuals, EBV infection is kept under control by CD8+ cytotoxic T-cells, which kill off the EBV-infected lymphoblastoid cell lines (38). Cell-mediated immune mechanisms, involving T and NK cells, are of pivotal importance in controlling the proliferation of EBV-infected B cells. Since specific cytotoxic CD8+ cells are primed to recognize and eliminate infected cells which present latent proteins of EBV, hereafter are referred to as latency-specific T-cells (39). The mechanisms leading to tolerance in the majority of individuals versus the induction of autoimmunity and disease in others are not even rudimentarily understood.

Several studies have used synthetic EBV peptides to investigate T Cell immunity to EBV in MS, with conflicting results. Studies using panels of HLA class I restricted EBV peptides have found an increased frequency of reactive CD8 T-cells in MS patients, in CIS but not established MS, or no increase in either CIS or MS patients. In one study, MS patients had an increased CD4 T cell response to peptides derived from EBNA-1 (40, 41, and 42).

EBV control relies to a large extent on T cells and NK cells. It could therefore be hypothesized that MS patients have a deficiency in the cellular immune control of EBV and possibly also other viruses. CD8 T cell infiltration of MS brain lesions has been demonstrated in several studies but defective T cell control of EBV has also been reported in MS patients. This could indicate an imbalance in the T cell control of EBV in MS patients, and one study has actually found increased programmed death (PD) 1 on CD8 T cells resulting in decreased cytolytic activity against EBV-infected B cells, while PD1 has also been reported to be increased on regulatory T cells (43).

A scenario referred to as Pender's hypothesis is that EBV may infect autoreactive B lymphocytes, which would become latently infected B memory cells that could circulate to the organ in which their antigen is expressed and act as antigen-presenting

cells for autoreactive CD4+ T cells. The thus activated autoreactive CD4+ T cells would then cause the actual organ damage in MS but also other autoimmune diseases associated with EBV (44).

Vaccination

Vaccinating against EBV could be vaccinating against MS. Nevertheless, due to the long incubation period, trials demonstrating that EBV vaccination in early childhood abrogates MS in later life appear challenging. It should also be noted that EBV vaccines that would not prevent EBV infection but rather delay it to an older age might be harmful, given the increased risk of MS associated with EBV infection later in life. Still, though there is currently no approved EBV vaccine available, a prophylactic EBV vaccine could be a means for primary prevention of MS (45).

MS and personalized medicine

The therapeutic approach to multiple sclerosis (MS) requires a personalized medicine frame beyond the precision medicine concept, which is not currently implementable due to the lack of robust biomarkers and a detailed understanding of MS pathogenesis. Personalized medicine demands a patient-focused approach, with disease taxonomy informed by characterization of pathophysiological processes. Important questions concerning MS taxonomy are: when does MS begin? When does the progressive phase begin? Is MS really two or three diseases? Does a therapeutic window truly exist? Newer evidence points to a disease spectrum and a therapeutic lag of several years for benefits to be observed from disease-modifying therapy. For personalized treatment, it is important to ascertain the disease stage and any worsening of focal inflammatory lesions over time (46, 47).

CONCLUSION

MS has traditionally been regarded as an autoimmune disease. However, the occurrence of autoantibodies (AuAbs) in MS (e.g., myelin basic protein (MBP) and major oligodendrocyte glycoprotein (MOG) Abs) is limited to only some patients and the pathogenic role of AuAbs remains debatable, while the search for autoantigens (AuAgs) in MS continues (48). There is convincing epidemiological evidence that EBV infection is a strong risk factor for MS development, although the mechanisms remain elusive. The epidemiological data suggest that MS risk could be markedly reduced by preventing EBV infection, which could only be possible with a hypothetical vaccine that confers permanent sterile immunity against EBV or, less effectively, by causing an iatrogenic EBV infection in early childhood, when the adverse effect of infection on MS risk seems mitigated (49). Assuming EBV

really acts as a cofactor in the pathogenesis of MS, there might be an opportunity for preventive strategies such as vaccinations. Hopefully one day, the following statement of Pierre Marie will become a reality: “I have little doubt, in fact, gentlemen, that in the employment of such a substance as the vaccine of Pasteur or lymph of Koch the evolution of insular (multiple) sclerosis will someday be rendered absolutely impossible” (50).

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