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New Evidence for EBV Infection as a Cause of Multiple Sclerosis

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The link between infections and autoimmunity is complex, comprising genetic and environmental interactions. Different infectious agents (viruses, bacteria, fungus and parasites) can trigger autoimmunity, which can be both pathogen and disease-specific. Genetic susceptibility, or an underlying immune dysregulation, may explain why only a subgroup of individuals develops autoimmunity after infections[1]. A number of neuroimmune disorders are known to occur in close timing to viral infection; examples are anti-NMDA receptor encephalitis occurring in children after recovery from Herpes simplex virus encephalitis[2], primary CNS vasculitis after Varicella-zoster virus infection[3], and most recently the distinct cytokine mediated neurological syndrome seen in children following SARS-CoV-2 infection[4]. In addition, infections during childhood may be relevant to the risk of autoimmunity in adulthood[1]. Post-infection autoimmunity can result from a number of

mechanisms, either in isolation or in combination, such as molecular mimicry, bystander activation, epitope spreading and polyclonal activation of B cells[5].

The inter-relationship between infection and neuroimmune disorders has been studied in multiple sclerosis (MS). There is a strong epidemiological association between Epstein-Barr virus (EBV) and MS in both adults and children[6]. Evidence of previous EBV infection is detected in more that 95% of adults worldwide. Infection with EBV usually occurs in childhood in poorer settings and is often delayed to adolescence and young adulthood in developed countries. Primary EBV infection may cause infectious mononeucleosis and, more rarely, haemophagocytic lymphohistocytosis (HLH), due to a virus-induced overactivation of the bone marrow cell cytokines. Following infection (which is frequently asymptomatic), EBV persists in a latent form in B-lymphocytes, and has been associated with various malignant tumours (e.g., Burkittt's lymphoma), and immune diseases (such as systemic lupus erythematosus)[7].

The high prevalence of previous EBV infection among individuals without MS has required very large epidemiological studies to demonstrate a link between EBV infection and MS. In a large metanalysis of 1005 MS cases, only 8 were classified as EBV seronegative (<1%), vs 103 of the 1,060 controls (10%).[8] In a paediatric study of 110 children with relapsing demyelination (of which 62 were RRMS and 48 non-MS relapsing demyelination, including Myelin oligodendrocyte glycoprotein antibody-associated disease and Aquaporin-4 antibody neuromyelitis optica spectrum disorder) all MS patients tested showed evidence of remote EBV infection compared to 42.9% of non-MS patients.[9]

To further explore whether EBV infection is a possible cause of MS, in the January issue of *Science*, Bjornevik et al[10] identified 955 cases of MS in a cohort of >10 million active-duty US military personnel over a 20-year period (1993-2013). For each case, the authors identified up to three samples collected before MS onset (the first available, the last before MS onset, and one in between) and stored in the Department of Defense Serum Repository. Cases were matched to two randomly selected individuals without MS. 801 MS cases and 1566 non-MS controls were identified, and their EBV infection status was assessed in the available samples.

Of the 801 cases who developed MS, 35 (4.4%) were EBV seronegative in the first sample. All but one of these EBV-negative MS cases (n=34, 97%) became EBV seropositive before the onset of MS. Of the 1566 individuals who did not develop MS, 107 (5.7%) were EBV negative, and 57% of these showed EBV seroconversion. The risk of MS increased 32-fold with EBV seroconversion by the time of the third sample versus persistent EBV seronegativity. The median time from first EBV-positive result to MS onset was 5 years (range 0-10). To test the hypothesis that the association between EBV infection and MS is not simply due to an increased predisposition to both infection and MS, a similar analysis was performed for cytomegalovirus (CMV), which showed a similar rate of CMV seroconversion between those who developed MS over time and those who did not. Furthermore, in an agnostic comparison of antibody response to the entire human virome between 30 MS cases and 30 matched controls, the overall antibody response to known viruses was similar between cases and control before and soon after symptom onset, with the exception of EBV, which argues against a general susceptibility to infection in MS. Serum levels of neurofilaments light chain, a marker of neuroaxonal degeneration and

inflammatory activity in the central nervous system[11], were not different between MS cases and controls, suggesting that the EBV seroconversion proceeds not only the MS diagnosis but also detectable tissue injury.

This study is the first to show evidence of a direct causality and implies that some individual who developed MS after EBV infection would not have developed MS if they had not been infected with EBV. The strengths of this study include: the longitudinal design of a very large cohort of individuals, which creates a 'natural experiment', where some people will show a EBV seroconversion during the follow-up, and some will not; the analysis of CMV infection status, which represents a negative control, especially because the risk and distribution of CMV infection in the general population are similar to those of EBV infection; the inclusion of the human virome-wide screening, whose results point against the theory that an immune dysregulation pre- or post-MS onset may increase susceptibility to MS. The increased risk of MS associated with EBV seroconversion is so high that no other genetic or environmental factor can explain it.

This study calls for future research to replicate this finding in a cohort of individuals who are younger that these US military personnel or even children, as at the age of the first sample, the majority of this cohort was already EBV positive, thereby reducing the number of seronegative cases who were followed up over time. Future studies will clarify whether EBV not only causes MS, but also MS activity, and whether the success of anti-CD20 therapy in reducing disease activity in MS could be partially explained by depleting circulating memory B-cells, the primary site for persistent latent EBV infection. Further studies, correlating EBV viral load with MS disease activity may indicate if individuals with MS fail to regulate the

normal symbiotic relationship between EBV and host immune cell homeostasis and if there is a role for antiviral treatment as disease modifier in MS. Finally, further insights into the reasons why few individuals who become infected with EBV, do not develop MS, and the rare cases of those who are persistently seronegative, but develop MS (assuming that MS is not a misdiagnosis), are going to provide the missing pieces in the 'puzzle' of MS pathogenesis.

From a public health point of view, an EBV vaccine is urgently needed to prevent the development of MS in people who need the EBV infection to develop it. The complexity of the EBV life cycle, the lack of appropriate animal models, and the limited reports on adjuvant selection and immune responses have hampered the development of a vaccine. As the Moderna Phase I EBV vaccine trial (mRNA-1189) opens this year, new vaccine development techniques may overcome some of the challenges and reduce the costs of both vaccine development and vaccination programs. Considering the long latency of EBV, it is likely that childhood vaccination will be required, so it may take decades until we can detect the impact of any EBV vaccination program on the incidence of MS.

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