BRAIN
SCIENTIFIC COMMENTARY

EBV-specific T-cell responses are telling us something important about multiple sclerosis

This scientific commentary refers to 'Broader anti-EBV TCR repertoire in multiple sclerosis: disease specificity and treatment modulation' by Schneider-Hohendorf *et al.* (https://doi.org/10.1093/brain/awae244).

The evidence that Epstein-Barr virus (EBV) plays a causal role in the development of multiple sclerosis (MS) is overwhelming; EBV infection is necessary but insufficient to develop MS. However, how EBV infection causes MS is unclear. The 'hit-and-run theory' proposes that EBV triggers autoimmunity through molecular mimicry. In contrast, the 'driver theory' posits that EBV, by continually cycling through its latent and lytic infection phases, drives MS disease activity and pathology via multiple mechanisms. These may include direct CNS infection[1]; stimulation of autoreactive T and B cells via molecular mimicry or bystander effects[2]; providing pro-survival signals to autoreactive B cells[3]; or upregulating a second pathogenic virus,[4] for example, a human endogenous retrovirus or human herpesvirus 6, which in turn causes further tissue damage.

In this issue of *Brain*, Schneider-Hohendorf and co-workers[1] investigate the association between EBV and MS by comparing EBV-specific T-cell responses in patients with MS to those of individuals with other neurological conditions. Their findings reveal a unique EBV-related T-cell response in MS that is modifiable by specific disease-modifying therapies (DMTs).[1] These new observations, together with anecdotal evidence that MS may respond to antiviral therapies that target EBV and/or retroviruses[2] and the fact that most licensed MS DMTs target memory B cells (where latent EBV resides),[3] support the driver theory, i.e. that replication of EBV is central to its role in MS pathogenesis (Fig. 1).

When individuals are exposed to a lytic primary EBV infection, or to reactivation of the virus from its latent pool, the innate immune system responds rapidly to control the infection. This initial response subsequently stimulates the adaptive immune system, which consists of B cells that produce antibodies, and T cells that are superficially divided into overlapping CD8+ and CD4+ subsets with different effector functions.

People with MS and those who are at higher risk of developing MS exhibit increased antibody titres and a broader antibody response to EBV epitopes when compared to healthy controls and those with other diseases.[4] EBV-specific antibodies, produced by plasma cells, circulate in bodily fluids to neutralize the virus or limit the effects of reactivation. In contrast, T cells require cell–cell contact. Once an EBV infection has been subdued, central memory T cells are produced and are primed to manage future viral reactivation or lytic infection (Fig. 1).

Intermittent lytic EBV reactivation allows the virus to infect salivary epithelial cells, resulting in viral shedding into the saliva, which is the predominant route for EBV transmission. As with most viruses, including SARS-CoV-2, quantitative and qualitative antibody and T-cell responses to EBV can potentially be used as immunological biomarkers of viral activity: higher antibody titres and greater numbers of EBV-reactive T cells in peripheral blood suggest recent lytic viral activity.[1] An analogy for this would be booster vaccination: every time EBV reactivates, it boosts or stimulates the immune response, increasing antibody titres and facilitating the development of a broader antibody repertoire.[4] The more expansive T-cell receptor (TCR) repertoire observed by Schneider-Hohendorf and colleagues[1] in people with MS, particularly for non-immunodominant viral epitopes, suggests that a greater immunological response is required to keep the virus under control in this population.

Studies using older technologies have shown that people with MS have a more expansive EBV-reactive T-cell repertoire against EBV nuclear antigen-1 (EBNA-1), including non-immunodominant epitopes, compared to healthy controls and people with other diseases.[5] Deep TCRβ sequencing and in silico analysis now allow researchers to analyse the entire TCRβ repertoire and determine whether a particular T cell is reactive to EBV and, if so, to which antigen, using publicly available databases.[1]

The current authors have previously shown that within pairs of identical twins discordant for MS, affected individuals have a broader EBV-specific TCRβ repertoire than their unaffected counterparts, a comparison that avoids confounding by genetic factors.[6] The TCRβs identified were reactive to both latent and lytic EBV antigens, implying that latent-lytic cycling of EBV boosts T-cell memory and its diversity in people with MS (Fig. 1). Individuals treated with natalizumab had an even broader EBV-reactive TCRβ repertoire, whereas this was not observed in association with interferon-beta or anti-CD20 therapies.[6] In their newer longitudinal study, the authors found that ocrelizumab, teriflunomide and dimethyl

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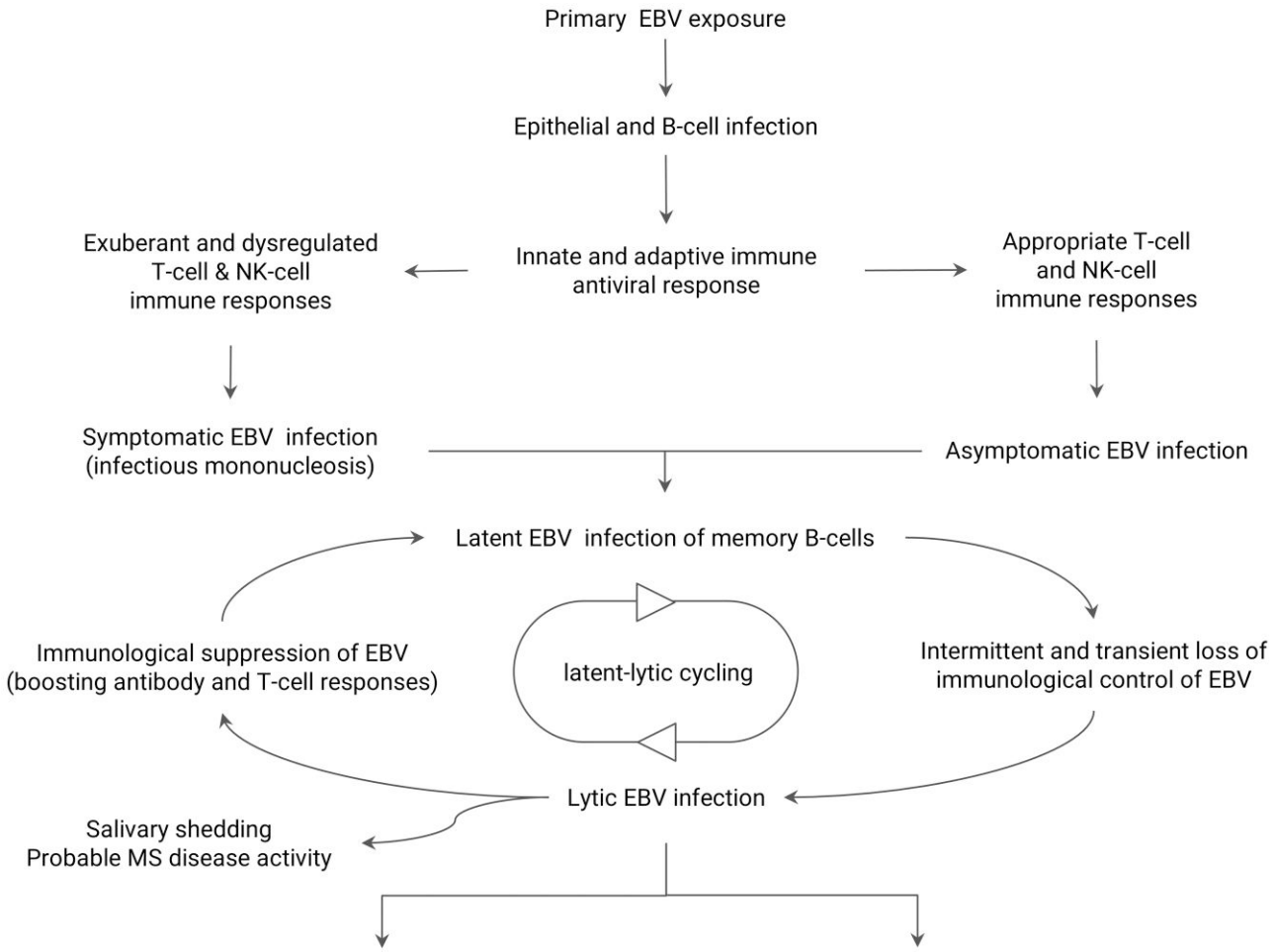

Multiple sclerosis	Normal and/or disease controls
Rapid dysfunctional EBV latent-lytic cycling	'Normal' EBV latent-lytic cycling
Higher and broader EBV antibody responses to both latent and lytic antigens	Lower and narrower EBV antibody responses to mainly latent antigens
Higher frequency and broader repertoire of EBV-specific T-cell responses to both latent and lytic antigens	Lower frequency and narrower repertoire of EBV-specific T-cell responses to mainly latent antigens
Epitope spread (B-cell and T-cell response to immunodominant and non-immunodominant EBV epitopes)	No epitope spread (B-cell and T-cell response to immunodominant EBV epitopes)
CSF central memory EBV-specific T-cells; i.e. recent priming (recent encounter with EBV in CNS)	CSF effector memory EBV-specific T-cells (no encounter with EBV in CNS)
EBV-specific CD8+ T-cell exhaustion	Normal EBV-specific CD8+ T-cell responses
Rejuvenation of EBV-specific CD8+ T-cell responses post-AHSCT	

Figure 1 Immunological control of Epstein-Barr virus (EBV) in people with multiple sclerosis compared to controls. AHSCT = autologous haematopoietic stem-cell transplantation; NK = natural killer; MS = multiple sclerosis.

fumarate reduced EBV-specific, but not cytomegalovirus (CMV)-specific, TCRβ sequences.[1] This implies that these three agents may work by suppressing latent-lytic cycling of EBV.

A recent Austrian study provided further insights into the role of EBV in MS, identifying genetic and immunological interactions that increased MS risk. The study showed that cytotoxic natural killer cells and distinct EBV-specific T cell responses were able to kill putative pathogenic autoreactive glial cell adhesion molecule-reactive cells ($GlialCAM_{370-389}$).[7] This response was genetically determined and was induced by EBV-variant-specific upregulation of the immunomodulatory HLA-E.[7] Host- and virus-defined genetic predispositions were associated with an up to 260-fold increased risk of developing MS.[7] In a subsequent study, the same group extended these findings to show that high levels of cross-reactive antibody responses between $EBNA_{381–452}$ and $GlialCAM_{370–389}$, and epitopes from three other putative MS-associated autoantigens—alpha-B-crystallin, myelin basic protein and anoctamin-2—were associated with a more than 1000-fold increased risk of MS.[8] These extraordinary results should be interpreted with caution as they have yet to be reproduced.

The overall picture that is emerging is that people with MS appear to have a problem controlling EBV from an immunological perspective. This is supported by work suggesting that EBV-specific cytotoxic T-cell responses are exhausted in MS, leading to poor virus control and greater MS disease activity.[9] This may also explain why patients with MS can respond well to autologous haematopoietic stem-cell transplantation (AHSCT), which invariably results in EBV reactivation to a greater or lesser extent. Schneider-Hohendorf and colleagues[1] performed TCRβ repertoire sequencing in two MS cohorts that underwent AHSCT. EBV-specific CD8+ T-cell responses peaked at 2 months after AHSCT in subjects with either EBV reactivation or EBV reinfection and returned to lower, but not baseline, levels after 12 months. These findings are consistent with other high throughput TCR analyses that detected expansion and diversification of the CD8+ cytotoxic T-cell responses to both EBV lytic and latent antigens 6 to 24 months after AHSCT.[10] Immune reconstitution in the presence of EBV reactivation likely rejuvenates jaded, exhausted or ineffective EBV-specific T-cell responses, enabling the control of EBV after AHSCT.[10]

In their earlier study, Schneider-Hohendorf and colleagues[1] found that in healthy individuals, EBV-specific T cells in peripheral blood and CSF consisted mainly of effector-memory cells.[6] In contrast, in subjects with MS, the CSF contained EBV-specific central-memory T cells,[6] indicating that these cells were recently primed or had recently been exposed to EBV antigens. For cells in the CSF, this is likely to have occurred in the CNS.

In summary, the findings from this latest TCRβ repertoire study[1] are consistent with an exaggerated anti-EBV immune response in MS and strongly suggest that EBV latent-lytic cycling drives MS disease activity. The data from natalizumab-treated patients and detailed analyses of T cells in the CSF indicate that EBV is likely active within the CNS in people with MS. The rejuvenation of EBV-specific T-cell responses after AHSCT may be one of the mechanisms underlying the therapeutic effectiveness of this procedure. Finally, the data support the exploration of CNS-penetrant EBV antivirals and EBV immunotherapies as treatments for MS.

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