EBV PRESENCE IN THE BRAIN OF PERSONS WITH MULTIPLE SCLEROSIS: NEW EVIDENCE USING RNASCOPE TECHNOLOGY

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The association between EBV and multiple sclerosis (MS) is one of the best documented pathogen-chronic disease associations. Epidemiological evidence is now available of a causal role for EBV in MS opening the possibility to target the virus for disease treatment and prevention [1]. Research is ongoing on the mechanisms leading EBV infection to cause immune-mediated central nervous system (CNS) inflammation in susceptible individuals. Based on the results of our own studies, performed across 15 years in postmortem brain tissue from cases with MS, we have proposed a model of MS pathogenesis that posits a persistent EBV infection in the CNS and a dysfunctional CD8 T cell response towards EBV as the main determinants of chronic neuroinflammation and bystander demyelination/neurodegeneration. This immunopathological model of MS is corroborated by two sets of data [2]. On the one hand, we have shown that both EBV latently infected B cells and EBV transcripts can be detected in MS lesions and meningeal ectopic B-cell follicles, that EBV reactivation associates with magnitude of inflammation and lesion activity in the MS brain, and that EBV dysregulation in the CNS is characteristic of MS and not of several other neuroinflammatory diseases. On the other hand, we have visualized contacts between cytotoxic CD8 T cells and B cells or EBV lytically infected B cells, and have shown preferential accumulation of EBV-specific CD8 T cells in the pathological tissue. Because over the years there have been conflicting findings on EBV presence in the MS brain, possibly due to technical issues and differences in tissue sampling, it is important to resolve this controversy using highly sensitive and specific assays, such as RNAscope in situ hybridization.

Here, we have performed RNAscope for EBER1 to assess EBV infection in postmortem brain samples from three progressive MS cases provided by the UK MS Tissue Bank at Imperial College. Experiments performed in sections of a tonsil from a case with EBV+ infectious mononucleosis as positive control allowed to visualize a red staining by EBER1 molecules in numerous cells localized in the subepithelial and perifollicular areas, while control lymph node and tonsil tissues were negative. In MS brain sections, selected for the presence of immune infiltrates and B cells, RNAscope allowed to detect EBER1 molecules in several lymphocyte-like cells within the perivascular space of some infiltrated blood vessels associated with white matter lesions and, more frequently, in B cell-rich infiltrates in the subarachnoid space lined by the pia mater and arachnoid meningeal layers. EBER1 positivity was detected as a red uniform or punctuated staining in different cells. Despite these encouraging results, further work is needed to establish the optimal conditions to use RNAscope ISH assays in autoptic MS tissues, and for the detection of different EBV latent and lytic transcripts, aiming to characterize latent and replicative EBV infection in the lesioned parenchyma and meninges.

In conclusion, we report here preliminary findings using RNAscope confirming EBV infection in postmortem MS brain samples. The results obtained indicate that this technique, enabling in situ RNA detection with single-molecule sensitivity, may prove useful to further investigate EBV dysregulation in the MS brain and verify the hypothesis that an EBV-induced immunopathological response plays a role in the development and maintenance of neuroinflammation and neurodegeneration.

1) Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, Elledge SJ, Niebuhr DW, Scher AI, Munger KL, Ascherio A. (2022) Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 375:296-301.

2) Veroni C, Aloisi F. (2021) The CD8 T Cell-Epstein-Barr Virus-B Cell Trialogue: A Central Issue in Multiple Sclerosis Pathogenesis. Front Immunol. 12:665718. doi: 10.3389/fimmu.2021.665718.