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ABSTRACT

EBV-Infected Lymphoblastoid Cell Lines: A Comparison of MS to Controls

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Background: Epstein-Barr virus (EBV) infection has long been associated with multiple sclerosis (MS). Essentially all people with MS are infected with EBV, and high levels of antibodies to the EBV nuclear antigen in healthy young people increase risk of MS. Although the association of EBV and MS is well established and the immune response against EBV correlates with MS disease activity, we don't know the mechanism by which EBV infection causes MS. Any mechanism for the role of EBV in MS should explain why only a small minority of EBV-infected people develop MS. Our hypothesis is that EBV-infected cells in people with MS are fundamentally different than EBV-infected cells in people who do not develop MS, and the differences are ones that would enhance or drive the autoimmune process in MS. The purpose of this study was to compare in vitro characteristics of EBV-infected cells between people with MS and healthy controls (HC).
Methods: EBV-infected lymphoblastoid cell lines (LCL) were previously collected and stored in liquid nitrogen. A total of 24 cell lines, 12 MS and 12 control, were thawed and grown in media. To assess the in vitro growth rate of LCL, cell numbers were counted using the Invitrogen Countess II automated cell counter every 48-72 hours, normalizing the growth rate for each cell line per 24 hours. To compare expression of surface molecules necessary for the B cells to cross the blood brain barrier, flow cytometry was performed by staining 5e5 cells with a panel of antibodies and running them through the Cytoflex B cytometer. Cell culture supernatant was collected after 72 hours of growth to measure Immunoglobulin G using ELISA. Quantitative PCR and quantitative reverse-transcriptase PCR were used to measure viral DNA and RNA for latent and early lytic transcripts expressed in the cells. To quantify the results of the PCR, Namalwa cells were grown and analyzed alongside the LCL cells.
Results: The equation, growth rate=$x^{(24/y)}$ given x=$cells^{final}/cells^{initial}$ and y=time of growth in hours, normalized the growth rate for each cell line per 24 hours. Growth rate in cells/mL and total concentration did not significantly vary between control and MS. Flow cytometry experiments showed some variation in surface marker expression, with focus on CD54, CD27, and CD274. The ELISA results obtained indicated high variability in IgG secretion between individual cell lines, and showed a trend of increased IgG secretion from MS. RT-qPCR analysis suggests a difference between EBER and BLLF1 expression in MS vs HC. EBER expression was higher in HC, indicating more HC cells were in the latent phase than MS. BLLF1 expression was higher in MS, and BLLF1 is considered pivotal in EBV infection of B cells.
Conclusion: EBV-infected lymphoblastoid cell lines from the 12 MS patients showed greater expression of the viral glycoprotein BLLF1 and less expression of the EBV-encoded small RNAs, EBER. Preliminary flow cytometry data indicates a possible difference in CD274, suggesting the MS LCL might stimulate the immune system more than the HC LCL. If this holds up in future analysis, it will indicate a new LCL phenotype in MS, and any consistent difference between MS and HC would be novel. The results from this study warrant future investigation into cell

surface marker expression and EBER and BLLF1 differences between MS and controls.