DETECTION OF EBV NUCLEIC ACIDS IN BRAIN IMMUNE INFILTRATES, CEREBROSPINAL FLUID AND PERIPHERAL BLOOD OF PATIENTS WITH MULTIPLE SCLEROSIS

Veroni C.[1], Marnetto F.[2], Valentino P.[2], Bertolotto A.[2], Frau J.[3], Cocco E.[3], Repice A.[4], Ballerini C.[5], Fagnani C.[1], Rosicarelli B.[1], Serafini B.[1], Aloisi F.[1]

[1] Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy; [2] CRESM (Multiple Sclerosis Regional Reference Center), Neuroscience Institute Cavalieri Ottolenghi (NICO) and AOU San Luigi Gonzaga, Orbassano (TO), Italy; [3] Department of Medical science and public health, University of Cagliari, MS Centre, Binaghi Hospital, Cagliari, Italy; [4] Department of Neuroscience, Drug and Child Health (NEUROFARBA), University of Florence, Italy; [5] Department of Experimental and Clinical Medicine (DMSC), University of Florence, Italy.

caterina.veroni@iss.it

Sero-epidemiological evidence strongly supports a causal role for EBV in multiple sclerosis (MS) providing new opportunities for disease therapy and prevention. In this perspective, quantification of EBV DNA and/or RNA in cerebrospinal fluid (CSF) and peripheral blood of MS patients could be useful for assessing disease prognosis or the response to therapy. To date, quantification of EBV DNA load in the CSF and peripheral blood has not revealed significant differences between healthy donors (HD) and MS patients, while analysis of MS brain tissue has provided conflicting results on the presence of EBV. Our studies using EBER ISH and immunohistochemistry for EBV proteins have unravelled accumulation of EBV latently and lytically infected B-lineage cells in the MS brain and, possibly, CNS draining lymphoid tissue.

Here, we compare data obtained in laser microdissected immune infiltrates from postmortem brain sections of chronic MS cases, CSF cells from MS patients, and PBMC from MS patients and HD using droplet-digital PCR (ddPCR) to quantify EBV BamHI-W copy numbers and preamplification real time RT-PCR to assess levels of transcripts associated with EBV latency (EBER1, EBNA3A, LMP1, LMP2A) and reactivation (BZLF1, gp350/220).

EBV transcripts were detected in a substantial proportion of immune infiltrates isolated from the brain of most MS cases analyzed (9/11), indicating profound local viral deregulation (latency II, latency III and/or reactivation]. EBV latency transcripts were detected more frequently than lytic transcripts (38.7 vs 6.6% of the samples; p=0.0001, Fisher's exact test) [1]. Analysis of CSF cells from therapy naïve patients with relapsing-remitting (RR) MS (n=101) revealed presence of EBV transcripts in only a small fraction of samples (7.9%). Interestingly, all 8 EBV+ CSF samples contained viral transcripts that are associated with EBV latency II, latency III and/or lytic cycle. The very low RNA amount extracted from CSF cells, together with the fact that B cells are a minor and variable CSF cell component, may explain absence of detectable EBV RNA in most samples. EBV DNA was detected in 2 out of 3 CSF samples that had enough cells to perform both RNA and DNA analysis; the viral load in CSF cells was approximately 10-fold higher than that in paired PBMC samples.

EBV nucleic acids were also analyzed in PBMC from therapy-naïve RRMS patients (n=79) and sex/age matched HD (n=45). EBV DNA load tended to be higher in MS (p=0.056, Mann-Whitney test), while EBV transcripts markedly differed between HD and MS patients. In HD, EBER1 alone was detected in 7 PBMC samples (15.6%) while 2 samples contained EBER1 and very low levels of LMP1 RNA (4.4%). In MS, EBER1 alone was detected in 6 PBMC samples (7.6%) while EBV transcripts associated with EBV latency II, latency III and/or lytic cycle were detected in 20 PBMC samples (25.3%), their prevalence being significantly higher in MS than in HD (p=0.002, Fisher's exact test).

In conclusion, highly sensitive and specific PCR-based techniques have allowed not only to confirm EBV presence in postmortem MS brain, corroborating the results of EBER ISH and immunohistochemical studies, but also to show for the first time dysregulation of EBV infection in CSF and peripheral blood cells of some RRMS patients.

1) Veroni C, Serafini B, Rosicarelli B, Fagnani C, Aloisi F. (2018) J Neuroinflammation 15(1):18