Specificity and phenotype of T cells in multiple sclerosis lesions.

Van Nierop, G.P.[1,2], Hintzen, R.Q.[2], and Verjans[1], G. M.G.M.

Department of [1]Viroscience and the [2]ErasMS centre at the department of Neurology, Erasmus MC,'s-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands.

Research question and background

The complex etiology of multiple sclerosis (MS) involves genetic predisposition and environmental factors, among which Epstein Barr virus (EBV) infection. Recent studies advocate the role of CD8 T-cells in initiation and perpetuation of MS pathology. We aim to characterize the phenotype and antigen specificity of both CD4 and CD8T-cells recovered from the cerebral spinal fluid (CSF), trigeminal ganglia (TG) and brain tissues of deceased MS patients.

Methods and tissues used

The acquisition of paired active white matter lesions, normal appearing white matter (NAWM), CSF, TG and blood with in general <6 hours post-mortem interval, offers the unique opportunity to compare the phenotype and specificity of CD8 T-cells derived from each anatomic location. Part of the brain tissues obtained were snap-frozen for future *in situ* analysis and the other part were dispersed for T-cell phenotyping using multicolor flow cytometry and generation of tissue-derived T-cell lines (TCL) using mitogenic stimulation. From paired blood samples, EBV-transformed B-cell lines (BLCL) were generated to be used as autologous antigen presenting cell in functional T-cell assays. Analogously, BLCL were used to stably express and present MS-associated autoantigens including MAG, MOG, PLP, KIR4.1, S100ß, CNTN2 and NF155. TCL generated from all specimens obtained were analyzed for clonal enrichment and T-cell reactivity towards both EBV and MS-associated autoantigens.

Results and conclusions

In situ analysis of MS lesions reveals extravasated T-cells, both CD4 and CD8 T-cells, in proximity of damaged areas expressing Ki67 and granzyme B, indicating local proliferation of antigen experienced T-cells. Flow cytometric analysis shows that intra-lesional CD8 outnumber CD4 T-cells and mainly express an effector memory phenotype. Interestingly, CD8 T-cells recovered from both lesions and NAWM show increased activation (e.g. CD95L, CD137 and ICOS) and inhibitory markers (e.g. PD-1 and TIM-3) compared to blood. However, T-cell receptor Vß serotyping shows a distinct oligoclonal repertoire in the paired NAWM and lesions for both CD4 and CD8 T-cells, again supporting a local antigen-specific T-cell response. Whereas no reactivity towards MS-associated autoantigen was detected, 2 of 7 patients analyzed showed elevated frequencies of BLCL reactive CD8 T-cells in MS lesions compared to control tissues suggesting the presence of EBV-specific T-cells in part of the MS lesions.