

Characterization of highly differentiated viral-specific T cells in multiple sclerosis using quantitative real-time PCR

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INTRODUCTION:

Several epidemiological studies have shown an association between Epstein-Barr virus (EBV), a γ -herpesvirus, and multiple sclerosis (MS) [1]. In contrast to the well studied EBV-specific humoral immune response in MS patients, the EBV-specific cellular immune response was less well characterized. Our group has recently found that there is a higher frequency of IFN- γ secreting EBV-specific CD8+ T cells in early MS patients (see poster of S. Jilek). These results are in line with results from Lünemann and Höllsberg, who respectively showed that EBNA1-specific memory CD4+ T cells are increased in MS patients [2] and that EBV-specific CD8+ T cells are found at an increased frequency in MS patients when compared to healthy controls [3]. Cytomegalovirus (CMV), another herpesvirus which has not been associated with MS, was used as a control in our study.

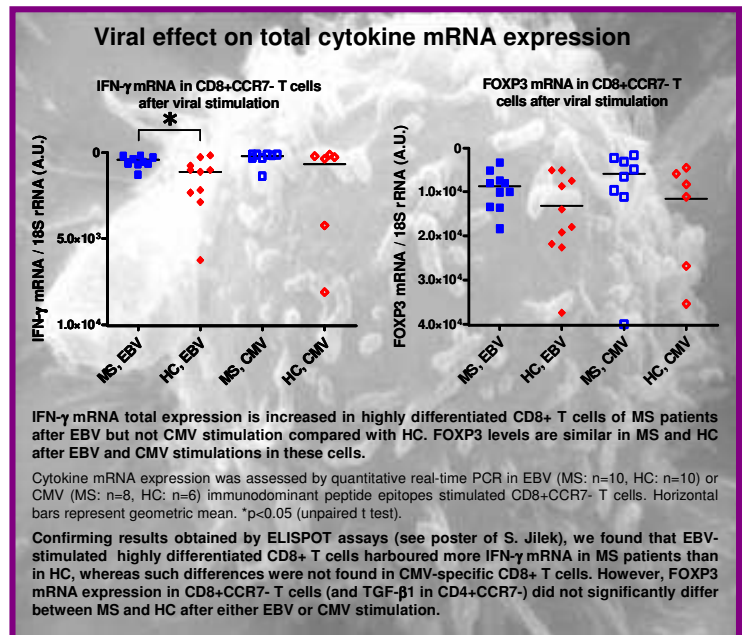
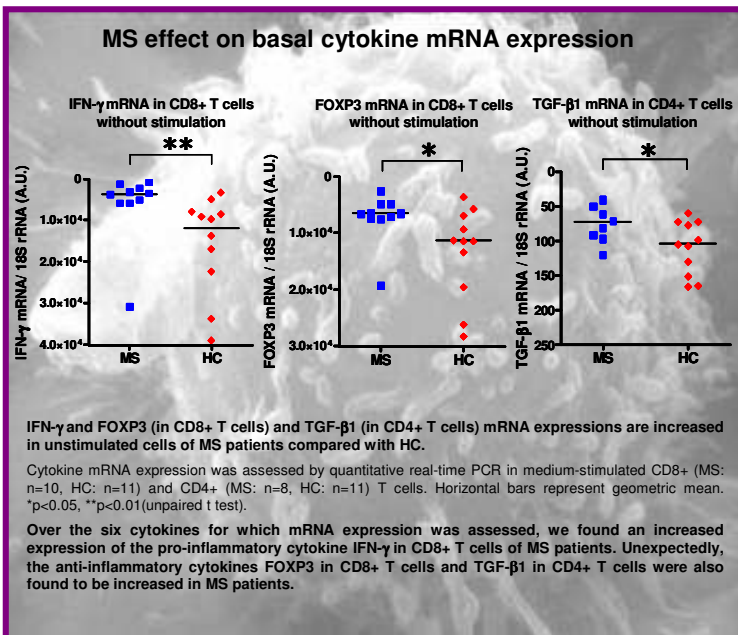
We have previously shown that highly differentiated (CCR7-) CD8+ T cells were enriched in the cerebrospinal fluid of early multiple sclerosis (MS) patients, thus arguing for a role of these cells at the onset of MS [4]. Therefore, in this study, we examined by quantitative real-time PCR (qRT-PCR) the cytokine mRNA expression under basal conditions in bulk CD4+ and CD8+ T cells and after EBV or CMV stimulation in highly differentiated CD4+ and CD8+ T cells. We used this method rather than intracellular cytokine staining since qRT-PCR is able to detect very low levels of these proteins in unstimulated lymphocytes.

MATERIAL AND METHODS:

Patients: We enrolled 10 inflammatory MS patients (3 patients with clinically isolated syndrome and 7 with relapsing-remitting MS) and 11 age-matched healthy controls (HC) with a strong EBV-specific T cell response. Of them, 8 MS patients and 6 HC were also identified to harbour a strong CMV-specific T cell response. All study subjects gave their informed consent according to the IRB of our hospital. After blood draw, PBMC were immediately processed.

Methods: We stimulated PBMC with either viral lysate or immunodominant peptide epitopes to elicit a CD4+ or a CD8+ response, respectively. After 17h of stimulation at 37°C, these T cells were sorted according to their CCR7 staining, RNA extracted from each cell subset, and cDNA synthesized. Expression of pro-inflammatory (IFN- γ , IL-2, IL-1 β) and of anti-inflammatory (FOXP3, IL-4, TGF- β 1) cytokines was assessed by qRT-PCR. 18S rRNA was used as an endogenous control to normalize for differences in the amount of cDNA between samples. Relative quantification of the transcripts was performed using the comparative 2^{- $\Delta\Delta$ Ct} method as described by Livak [5]. The difference of expression between target genes and the reference gene (18S rRNA) corresponded to the basal level of gene expression in medium-stimulated cells or to the total level of gene expression in virus-stimulated cells.

Statistics: Data were LOG-transformed before statistical analysis to render them normally distributed. Two-tailed unpaired t test was used.



CONCLUSION:

It is now widely accepted that EBV is linked to MS [1]. EBV-specific humoral and cellular immune responses were shown to be increased in MS patients [1, 2, 3, poster of S. Jilek]. On the other hand, dysregulation of cytokine secretion was recently demonstrated in MS patients [6, 7, 8]. However, the majority of these studies have been performed in the serum or PBMC of patients, thus limiting the identification of the cells involved.

Here, we show that basal IFN- γ mRNA expression is significantly more elevated in the CD8+ T cells of MS patients compared to HC, suggesting that CD8+ T cells are already more activated in MS before any stimulation.

Similarly, but more surprisingly, the anti-inflammatory cytokines TGF- β 1 and FOXP3 were increased in CD4+ and CD8+ T cells respectively. This elevated basal anti-inflammatory cytokine expression could be explained by the fact that all the patients except one were in remission or that there is a feedback mechanism trying to reduce the elevated IFN- γ basal expression.

Furthermore, we confirmed our previous results [poster of S. Jilek]. Indeed, we found a significantly higher IFN- γ mRNA expression after EBV stimulation in CD8+CCR7- T cells, but not in EBV-stimulated CD4+CCR7- or CMV-stimulated CD4+CCR7- and CD8+CCR7-. However, there was no difference in the expression of the other four cytokines (IL-2, IL-1 β , FOXP3, TGF- β 1) after EBV or CMV stimulation in our study subjects. This reinforces the link between EBV, IFN- γ secreting effector T cells and MS.

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