

# Strong EBV-specific CD8+ T cell Response in Patients with Early Multiple Sclerosis\*

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\* Jilek et al., Brain 2008, 131:1712

## INTRODUCTION:

Epstein-Barr virus (EBV), in contrast to CMV, has repeatedly been associated with multiple sclerosis (MS) [1-6]. However, if EBV is associated with MS, is it really a trigger of the disease or would it rather be an accompanying marker associated with the degree of activity of MS? In a previous study, we demonstrated that highly differentiated CD8+ T cells were recruited in the CSF of MS patients, reinforcing the role of CD8+ T cells at the onset of MS [7]. Therefore, in this study, in an attempt to answer the question of the involvement of EBV in early MS, we examined the specific cellular immune response against EBV in patients with different categories of MS, other neurological diseases (OND) and healthy controls (HC). We used CMV as a control virus. Using ELISPOT assays, we studied the EBV- and CMV-specific effector CD4+ and CD8+ T cells through their secretion of IFN-γ.

## MATERIAL AND METHODS:

**Patients:** We enrolled patients with clinically isolated syndrome (CIS) or definite relapsing-remitting- (RR), secondary-progressive- (SP), primary-progressive- (PP) MS and patients with other neurological disorders (OND) at the outpatient clinics. All patients gave their informed consent according to the IRB of our hospital. PBMC were isolated and immediately processed or frozen for further use. Sera of patients were tested for the presence of antibodies against EBV and seronegative patients were excluded from the study.

**ELISPOT:** To assess for the effector capacity of EBV-specific T cells, PBMC were stimulated with EBV viral lysate or pool of immunodominant EBV peptide epitopes. IFN-γ secretion was assessed after 18h of incubation at 37°C.

**Humoral response:** anti-EBV antibodies were measured with a bead-based multiplexed immunoassay (Luminex).

**Statistics:** Results were corrected for age over the whole cohort to eliminate this confounding factor. Kruskal-Wallis and Mann-Whitney-ranked tests for non-parametric data were used.

Table I. Clinical data of the 164 patients enrolled.

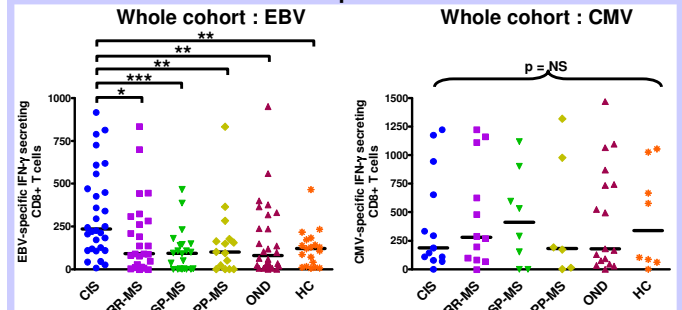
	Inflammatory MS				OND (n=35)	HC (n=21)
	CIS (n=35)	RR-MS (n=31)	SP-MS (n=24)	PP-MS (n=18)		
Age at blood draw (years)	39 ± 14	41 ± 7	57 ± 16	55 ± 7	39 ± 20	35 ± 10
Delay between disease onset and study entrance (years) <sup>2</sup>	0.4 ± 1.2	8.2 ± 6.7	14.9 ± 14.3	5.6 ± 5.4	0.4 ± 1.0	n/a
Patients in relapse	9	19	2	0	n/a	n/a
Patients in treatment	0	10	0	0	n/a	n/a
Number of MS diagnosis subsequently confirmed	18 (follow-up 2.4 ± 0.9 y)	n/a	n/a	n/a	n/a	n/a
EBV infection (%) <sup>3</sup>	100	100	100	100	97	100
CMV infection (%) <sup>3</sup>	46	43	61	50	65	40

<sup>1</sup>Numbers represent median ± interquartile range.

<sup>2</sup>Study entrance corresponded to the diagnostic procedure including drawing of blood sample for further assays.

<sup>3</sup>Numbers represent percentage of EBV, respectively CMV infection in the different patient groups.

## Elevated frequency of IFN-γ secreting EBV-specific effector CD8+ T cells in patients with CIS

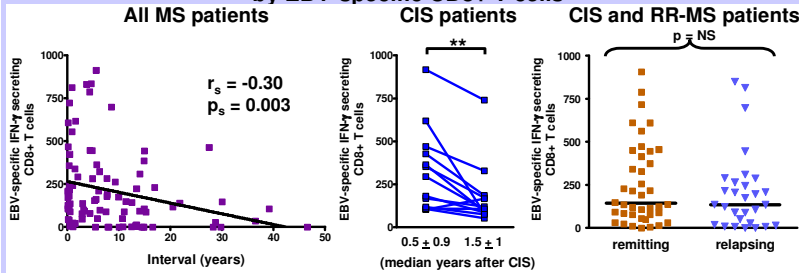


Increased frequency of IFN-γ-secreting EBV-specific effector CD8+ T cells in inflammatory MS patients.

IFN-γ secretion of CD8+ T cells was assessed in PBMC of patients with CIS (n=30), RR-MS (n=25), SP-MS (n=22), PP-MS (n=15), OND (n=28) and HC (n=19) after stimulation with EBV or CMV immunodominant CD8+ restricted peptides. Results are expressed as spot forming cells for 10<sup>6</sup> cells. Horizontal bars represent the median values. NS, non significant (Kruskal-Wallis ranked test); \*p<0.05, \*\*p<0.01, \*\*\*p<0.005 (Mann-Whitney ranked test).

In all patients, IFN-γ-secreting CD4+ T cells were less frequent than CD8+ T cells and no difference in frequency of effector CD4+ T cells between the groups was found (data not shown). However, patients with CIS display increased frequency of EBV-specific effector CD8+ T cells when compared to RR-MS, SP-MS, PP-MS, OND or HC.

## The more recent the clinical onset, the higher the level of IFN-γ secretion by EBV-specific CD8+ T cells



Interval between disease onset and assay but not activity of disease in MS patients is linked to EBV-specific effector CD8+ T cell response.

**Left panel:** EBV-specific CD8+ T cells response (IFN-γ) according to the interval between MS onset and the assay in inflammatory and chronic MS. Results are expressed as spot forming cells for 10<sup>6</sup> cells.  $r_s$ , Spearman's non parametric correlation;  $p_s$ , Spearman.

**Middle panel:** In 13 patients with CIS, secretion of IFN-γ by EBV-specific CD8+ T cells was assessed on two time-points separated by a median of 1.0 ± 0.2 years. Results are expressed as spot forming cells for 10<sup>6</sup> cells. \*\*p<0.01

**Right panel:** MS patients (CIS, RR-MS and SP-MS) were divided into two groups (no relapse and relapsing) depending on their disease activity. Horizontal bars represent the median values. NS, non significant (Mann-Whitney ranked test).

We found that there was no difference between the secretion of IFN-γ by EBV-specific effector CD8+ T cells in relapsing versus remitting patients. However, the difference of activity of EBV-specific CD8+ T cells in inflammatory patients (CIS/RR-MS) could be attributed to the interval between disease onset and assay.

## CONCLUSION:

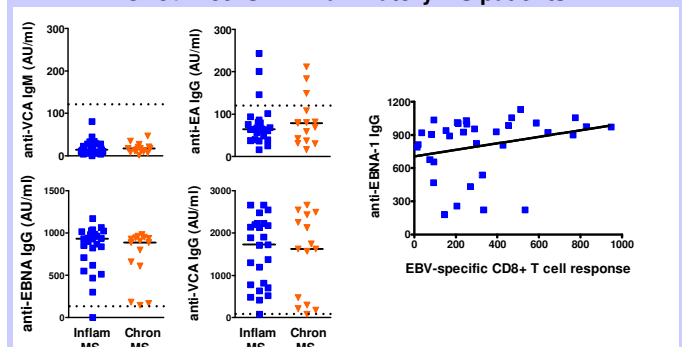
An association between EBV and MS has previously been shown [1,2]. Here, in an attempt to precise the putative role of EBV in the pathophysiology of MS, we performed a comprehensive study of the cellular and humoral immune responses against this virus. We found that EBV-specific effector CD8+, but not CD4+, T cells were significantly more elevated in patients with CIS (early MS) as compared to all other categories (RR-MS, SP-MS, PP-MS, OND and HC). In contrast, no difference in the CMV-specific T cell responses were found between the different categories of subjects. Furthermore, CMV responses were not dependent on the duration of MS, thus excluding a role for CMV at MS onset.

Interestingly, this high activation of EBV-specific effector CD8+ T cells was not attributable to the degree of activity of MS (relapses versus remission) but was inversely proportional to the duration of MS: the shorter the duration, the higher the magnitude of IFN-γ secretion by EBV-specific effector CD8+ T cells.

The humoral response specific for EBV was similar in all MS patients tested and was moderately correlated with the frequency of EBV-specific IFN-γ secreting CD8+ T cells in patients with inflammatory MS. However, EBV-specific CD8+ T cells are a better marker of early MS than EBNA-1 IgG antibodies.

In conclusion, we show here that EBV-specific effector CD8+ T cells are associated with early MS. These data are consistent with a role of EBV as a trigger of MS.

## Anti-EBNA-1 IgG are correlated with the secretion of IFN-γ by CD8+ T cells in inflammatory MS patients



Humoral response specific for EBV in inflammatory and chronic MS patients.

**Right panels:** Sera from 40 selected patients were collected and assayed for anti-VCA IgG, anti-EA IgG, anti-EBNA-1 IgG and anti-EBN-1 IgG using a bead-based multiplex array (Luminex). Data are presented as arbitrary units/ml and the dotted line determines the threshold for positive response. NS, non significant (Kruskal-Wallis ranked test).

**Left panel:** In patients with inflammatory MS (CIS/RR-MS), anti-EBNA-1 IgG were moderately correlated with the IFN-γ secretion by EBV-specific CD8+ T cells.  $p_s$ , Spearman's non parametric correlation;  $r_s$ , Spearman.

The magnitude of anti-EBNA-1, but not anti-VCA IgG, response was moderately correlated with the one of EBV-specific CD8+ T cells secreting IFN-γ in patients with inflammatory MS, but not in patients with chronic MS. However, in contrast to what we have found for IFN-γ secreting CD8+ T cells, there was no correlation between the level of anti-EBNA-1 IgG or anti-VCA IgG and the interval between MS onset and our assay.

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