

Strong EBV-specific effector CD8+ T cell Response in Patients with Early Multiple Sclerosis

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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 obtained 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.

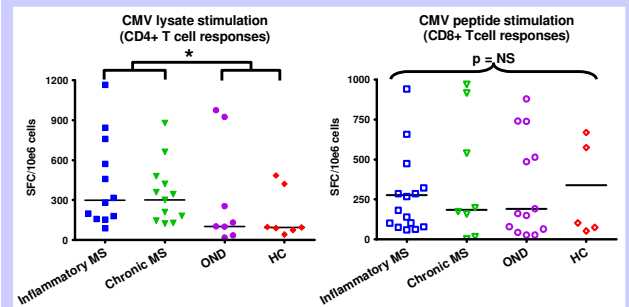
Statistics: Results were corrected for age over the whole cohort to eliminate this confounding factor. Kruskal-Wallis and Mann-Whitney-ranked tests for non-parametric data were used.

Table I. Clinical data of the 146 patients enrolled.

	Inflammatory MS		Chronic MS			OND	HC
	CIS (n=28)	RR-MS (n=28)	SP-MS (n=16)	PP-MS (n=18)	OND (n=35)		
Age at blood draw (years)	39 ± 15	39 ± 7	60 ± 16	53 ± 9	39 ± 20	35 ± 10	
Delay between disease onset and study entrance (years) ^{1,2}	0.4 ± 2.0	7.7 ± 7.4	14.7 ± 16.3	5.4 ± 5.3	0.4 ± 1.0	n/a	
Patients in relapse	6	17	2	0	n/a	n/a	
Patients in treatment	0	9	0	0	n/a	n/a	
Number of MS diagnosis subsequently confirmed	10 (follow-up 1.0 ± 1.2 y)	n/a	n/a	n/a	n/a	n/a	
EBV infection (%) ³	100	100	100	100	97	100	
CMV infection (%) ³	40	42	54	53	65	40	

¹Numbers represent median ± interquartile range.
²Study entrance corresponded to the diagnostic procedure including drawing of blood sample for further assays.
³Numbers represent percentage of EBV, respectively CMV infection in the different patient groups.

CMV-SPECIFIC EFFECTOR T CELLS

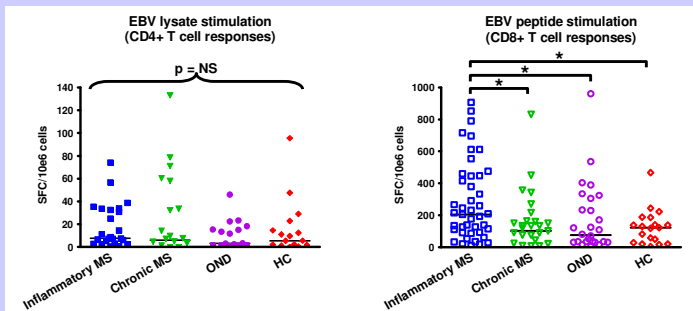


CMV-specific stimulation of effector CD4+ and CD8+ T cells leads to secretion of IFN- γ .

IFN- γ secretion of CD4+ and CD8+ T cells was assessed in PBMC of inflammatory MS (CIS/RR-MS; n=12), chronic MS (SP-/PP-MS; n=13), OND (n=9) and HC (n=7) after stimulation with CMV lysate or CMV immunodominant peptide epitopes. Horizontal bars represent the median values. NS, non significant (Kruskal-Wallis ranked test); *p<0.05 (Mann-Whitney ranked test).

■ Inflammatory MS (CIS/RR-MS); ▼ Chronic MS (SP-/PP-MS); ● OND; □ HC; SFC/10⁶ cells, spot forming cells for 10⁶ cells.
 The majority of patients responded to in vitro CMV stimulation. Interestingly, IFN- γ secretion by CD4+ T cells of MS patients (inflammatory and chronic) was higher than CD4+ T cells from OND or HC. However, this could merely be due to an aspecific immune hyper-activation. No difference between the categories for CD8+ effector T cells was found.

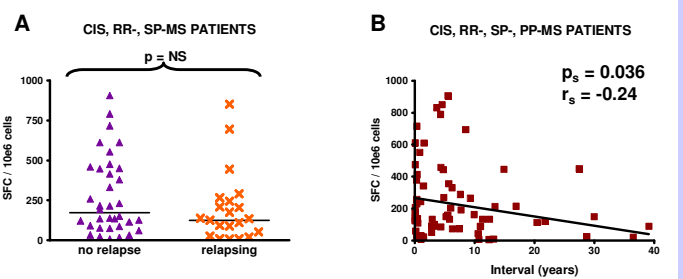
EBV-SPECIFIC EFFECTOR T CELLS



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

IFN- γ secretion of CD4+ and CD8+ T cells was assessed in PBMC of inflammatory MS (CIS/RR-MS; n=43), chronic MS (SP-/PP-MS; n=25), OND (n=25) and HC (n=19) after stimulation with EBV lysate or EBV immunodominant peptide epitopes. Horizontal bars represent the median values. NS, non significant (Kruskal-Wallis ranked test); *p<0.05 (Mann-Whitney ranked test).

■ Inflammatory MS (CIS/RR-MS); ▼ Chronic MS (SP-/PP-MS); ● OND; □ HC; SFC/10⁶ cells, spot forming cells for 10⁶ cells.
 In all patients, IFN- γ -secreting CD4+ T cells were less frequent than CD8+ T cells. Furthermore, no difference in frequency of effector CD4+ T cells between the groups was found. However, CIS/RR-MS patients, that is patients with inflammatory stages of the disease, display increased frequency of EBV-specific effector CD8+ T cells when compared to chronic MS patients (SP-/PP-MS), OND or HC.



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

A) 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.
 B) EBV-specific CD8+ T cells response (IFN- γ) according to the interval between MS onset and the assay in inflammatory and chronic MS. SFC/10⁶ cells, spot forming cells for 10⁶ cells; NS, non significant (Mann-Whitney ranked test); p_s, Spearman's non parametric correlation; r_s, Spearman r.

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 immune response against this virus. We found that EBV-specific effector CD8+, but not CD4+, T cells were significantly more elevated in patients with inflammatory MS (early MS) as compared to all other categories (chronic MS, OND and HC). On the other hand, CMV-specific T cell responses were probably due to an aspecific immune hyperactivation. Indeed, in contrast to EBV, 1) only 50% of our patients were infected with CMV, 2) there was no difference between inflammatory and chronic MS, and finally 3) CMV responses were not dependent on the duration of MS.

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.

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.

REFERENCES:

- Ascherio et al. (2007) *Ann Neurol* 61:288
- Haahr et al. (2006) *Rev Med Virol* 16:297
- Pohl et al. (2006) *Neurology* 67:2063
- Alotaibi et al. (2004) *JAMA* 291:1875
- Levin et al. (2005) *JAMA* 293:2496
- Thacker et al. (2006) *Ann Neurol* 59:493
- Jilek et al. (2007) *Clin Immunol* 123:105

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