

EBV-specific CD8+ T cells are more activated in patients with early MS

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INTRODUCTION:

Epstein-Barr virus (EBV) has repeatedly been associated with MS [1]. Seroepidemiological studies demonstrated that about 100% of MS adult patients are infected with EBV in contrast to healthy control subjects, which are infected at 90-95% [2]. Moreover, a significant higher rate of EBV infection was found in children with MS as compared to age-matched controls [3,4]. Prospective studies using serum samples collected before the onset of disease showed that the higher the anti-EBV antibody titers, the higher the risk of developing MS 10 years later [5]. A meta-analysis has shown a positive association between infectious mononucleosis, symptomatic EBV infection, and MS [6]. Contrasting with the abundant literature on the EBV-specific humoral immune response, few studies were published regarding the EBV-specific cellular one, in MS patients. EBNA1-specific memory CD4+ T cells were shown to be increased in MS patients [7] and an increased frequency of EBV-specific CD8+ T cells was found in MS patients when compared to healthy controls [8].

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 [9]. Therefore, in this study, we examined the specific cellular immune response against EBV in patients with MS, OND and healthy controls (HC). We studied the EBV-specific CD4+ and CD8+ T cell proliferative responses and IFN- γ secretion.

MATERIAL AND METHODS:

Patients: We enrolled patients with clinically isolated syndrome (CIS) or definite RR-, SP-, PP-MS and patients with 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.

Proliferation assays (PA): To determine the memory capacity of EBV-specific T cells, PBMC were stimulated with EBV lysate or pool of immunodominant EBV peptide epitopes and were assessed by proliferation assay using thymidine incorporation. The proliferation was assessed after 5 days of incubation at 37°C.

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.

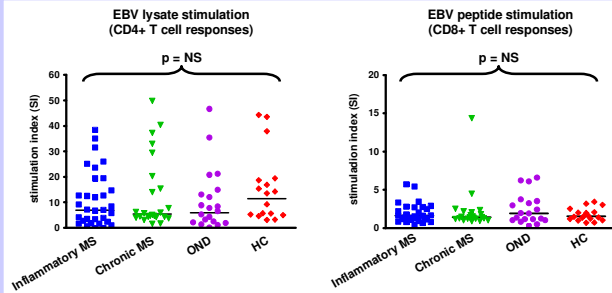
	Inflammatory MS		Chronic MS		OND (n=35)	HC (n=21)
	CIS (n=28)	RR-MS (n=28)	SP-MS (n=16)	PP-MS (n=18)		
Age at blood draw (years) ¹	39 ± 15	39 ± 7	60 ± 16	53 ± 9	39 ± 20	35 ± 10
Delay between disease onset and study entrance (years) ^{1,2}	0.4 ± 2.0	7.7 ± 7.4	14.7 ± 16.3	5.4 ± 5.3	0.4 ± 1.0	n/a
Patients 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

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 infection in the different patient groups.

MEMORY T CELLS



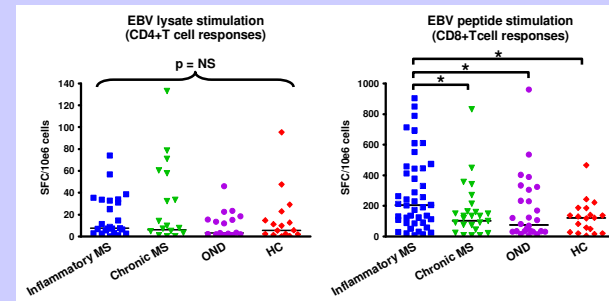
EBV-specific stimulation of memory CD4+ and CD8+ T cells leads to their proliferation as seen with proliferation assay

Proliferation of CD4+ and CD8+ T cells was assessed in PBMC of inflammatory MS (CIS/RR-MS; n=32), chronic MS (SP-/PP-MS; n=21), OND (n=21) and HC (n=16) after stimulation with EBV lysate or EBV immunodominant peptide epitopes. Horizontal bars represent the median values. NS, non significant (Kruskal-Wallis ranked test).

■ Inflammatory MS (CIS/RR-MS); ▼ Chronic MS (SP-/PP-MS); ● OND; □ HC.

The majority of patients responded to in vitro EBV stimulation. Overall CD4+ T cells had a higher proliferative capacity than CD8+ T cells. No difference in the proliferative capacity of CD4+ or CD8+ T cells was seen between the four groups of patients.

EFFECTOR T CELLS

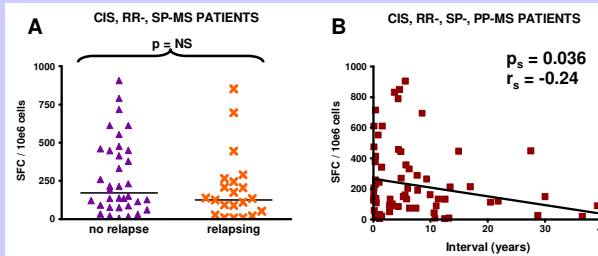


Higher frequency of IFN- γ -secreting EBV-specific effector CD8+ T cells in inflammatory MS patients using ELISPOT assay

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/10e6 cells, spot forming cells for 10e6 cells.

In all patients, IFN- γ -secreting CD4+ T cells were less frequent than CD8+ T cells. Furthermore, no difference in frequency of 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/10e6 cells, spot forming cells for 10e6 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:

It has previously been shown that EBV was linked to MS [1,2]. However, the majority of the studies assessed the EBV-specific humoral immune response. Here we show that EBV-specific CD8+, but not CD4+, T cells are significantly more elevated in patients with inflammatory MS as compared to all other categories (chronic MS, OND and HC).

Interestingly, this high activation of EBV-specific 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 CD8+ T cells.

In conclusion, we show here evidence that in EBV-specific CD8+ T cells are associated with early MS. Cross-recognition of EBV peptides and myelin proteins by the same TCR of a CD4+ T cell clone has recently been demonstrated [10]. By analogy, one could hypothesize that EBV-specific CD8+ T cells might be instrumental in triggering MS, for instance by a mechanism of molecular mimicry. Alternatively, EBV-specific CD8+ T cells might be a surrogate marker of early MS. Further studies are needed to precise the role of this virus in MS.

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