

Increased EBV reactivation in the cerebrospinal fluid of patients with early multiple sclerosis



Emilie Jaquiéry¹, Samantha Jilek¹, Myriam Schluep², Pascal Meylan³,
Andreas Lysandropoulos², Giuseppe Pantaleo¹, Renaud A. Du Pasquier^{1,2}

Divisions of ¹Immunology and ²Neurology, ³Institute of Microbiology, CHUV, 1011 Lausanne

INTRODUCTION

Epstein-Barr virus (EBV) has been consistently associated with multiple sclerosis (MS) [1-3]. Recently, EBV-infected B cells have been found in neo-follicles in the meninges of *post-mortem* patients with MS. Interestingly, activated CD8⁺ T cells were located in close contact with these EBV-infected B cells, strongly suggesting that the latter are recognized and attacked by CD8⁺ T cells [4]. Consistent with these data, we have shown that early MS patients harbor an increased EBV-specific, IFN- γ mediated, CD8⁺ T cell response in the blood [5], as well as an enrichment in highly differentiated CD8⁺ T cells in the cerebrospinal fluid (CSF) [6]. To test the idea that MS might be initiated by an abnormal EBV-specific immune response in the central nervous system, we examined the EBV-specific cellular and humoral immune responses in the CSF and blood of patients with early MS or other neurological diseases, separated into inflammatory (IOND) and non-inflammatory (NIOND) groups. The neurotropic herpesvirus cytomegalovirus (CMV) served as a control.

MATERIAL AND METHODS

Patients: We obtained paired blood and CSF samples of 60 patients referred to our Department of Neurology for investigations, including patients who had their first symptoms of MS less than one year prior to our assays (early MS) and patients with IOND and NIOND.

Effector cells: PBMC and CSF cells were stimulated with EBV- or CMV-specific pools of immunodominant peptide epitopes and cultured for 11-14 days in the presence of exogenous IL-2.

ICS: Effector cells cultured for 6-10 days were assessed for IFN- γ secretion in CD4⁺ and CD8⁺ T cells by FACS.

Functional CFSE CTL assay: Target cells were prepared by staining autologous PBMC with CFSE and loading them with EBV or CMV peptide pools. After 18h of incubation with increasing ratios of effector cells, surviving target cells were quantified by FACS and specific lysis was calculated.

Cytokine secretion assay: Effector cells were restimulated for 48h with peptide loaded feeder cells and cytokine secretion in the supernatant was assessed using a cytometric beads array.

Serologies: anti-EBV IgG were measured with a multiplexed immunoassay (Luminex) and anti-CMV IgG with an ELISA.

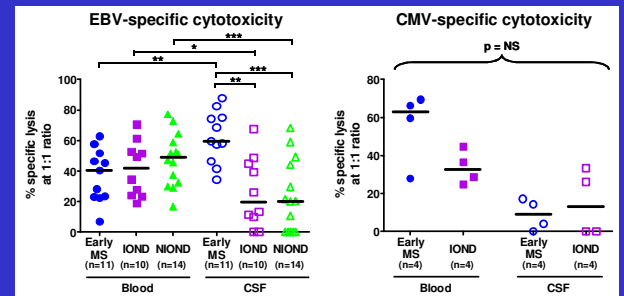
CLINICAL DATA OF THE COHORT

	Early MS (n=13)	IOND (n=15)	NIOND (n=16)
Age in years ¹	34 ± 11	42 ± 8	44 ± 18
Disease duration in months ¹	1.4 ± 4.2	1.1 ± 2.5	0.2 ± 0.3
CSF leucos per μ l ¹	4 ± 8	5 ± 3	1 ± 0.3
Intrathecal synthesis of IgG	12/13	5/15	0/16
EBV responders in blood ²	11/13	10/15	14/16
EBV responders in CSF ³	11/11*	8/10	9/14*
CMV responders in blood ²	4/9	4/8	0/1
CMV responders in CSF ³	2/4	2/4	n/a

¹Numbers represent median \pm interquartile range.
²Defined by an ICS assay with >1% of IFN- γ secreting CD8⁺ T cells.
³Defined by a CTL assay with >15% specific lysis at any effector:target ratio.
*Fisher's exact test: p<0.05

RESULTS

Enrichment in EBV-specific CD8⁺ CTL in the CSF of patients with early MS



EBV- and CMV-specific CD8⁺ CTL activities in the blood and CSF of study patients.

In patients with a positive ICS assay in the blood, determination of virus-specific CD8⁺ CTL activity in the blood and CSF was done using a functional CFSE CTL assay and effector cells stimulated with EBV or CMV immunodominant CD8⁺-restricted peptides for 11-14 days. Horizontal bars represent the median values. NS, non significant (Kruskal-Wallis ranked test); *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney or Wilcoxon ranked tests).

EBV-, but not CMV-specific, CD8⁺ CTL responses in the CSF of patients with early MS were significantly higher than in the CSF of patients with IOND, NIOND, as well as compared to paired blood. By contrast, the EBV-specific CD8⁺ CTL response was lower in the CSF than in the blood in patients with IOND and NIOND.

Elevated secretion of cytokines by EBV-specific CSF effector T cells of patients with early MS

		Blood			CSF		
		Early MS	IOND	NIOND	Early MS	IOND	NIOND
EBV	IFN- γ	5/5	6/6	8/8	11/11**	3/9**	3/12**
	TNF- α	0/5	0/6	2/8	5/11*	0/9*	0/12*
	IL-2	0/5	0/6	0/8	0/11	0/9	0/12
	IL-4	0/5	0/6	1/8	5/11*	1/9	0/12*
	IL-6	1/5	0/6	0/8	3/11	0/9	0/12
	IL-10	0/5	0/6	0/8	0/11	0/9	0/12
CMV	IFN- γ	3/3	3/3	n/a	1/4	2/4	n/a
	TNF- α	0/3	0/3	n/a	0/4	1/4	n/a
	IL-2	0/3	0/3	n/a	0/4	0/4	n/a
	IL-4	1/3	0/3	n/a	1/4	1/4	n/a
	IL-6	0/3	0/3	n/a	0/4	1/4	n/a
	IL-10	0/3	0/3	n/a	0/4	0/4	n/a

EBV- and CMV-specific cytokine secretion by blood and CSF effector cells of study patients.

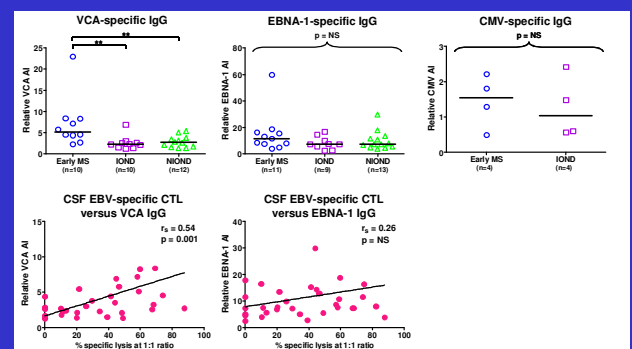
Cytokine secretion in the supernatants of effector cells restimulated for 48h with antigen-loaded feeder cells was determined using a multiplexed cytokine beads array. *p<0.05, **p<0.01 (Fisher's exact test).

IFN- γ (11/11 patients, range: 410-8759 pg/ml), TNF- α (5/11, 47-162 pg/ml) and IL-4 (5/11, 40-162 pg/ml) were more frequently secreted by EBV-specific CSF T cells of patients with early MS than those with IOND (IFN- γ : 3/9, 176-382 pg/ml; TNF- α : 0/9, and IL-4: 1/9, 125 pg/ml) or with NIOND (IFN- γ : 3/12, 337-393 pg/ml; TNF- α and IL-4: 0/12). The supernatants of CMV-stimulated CSF T cells contained little trace of cytokines, without difference between early MS and IOND patients.

CONCLUSIONS

- EBV-specific CD8⁺ CTL were enriched in the CSF of patients with early MS.
- The activity of these EBV-specific CD8⁺ CTL was significantly enhanced in the CSF as compared to the blood of patients with early MS.
- In addition to an enhanced cytotoxic activity, EBV-specific CD8⁺ T cells from the CSF of patients with early MS also displayed an increased secretion of IFN- γ , TNF- α and IL-4. These cytokines are known to play important roles in antigen-specific T cell responses.
- By contrast, there was no CSF recruitment of CMV-specific CD8⁺ CTL, nor was there any indication of an increased EBV-specific CD8⁺ CTL activity in patients with OND, but they inflammatory or not. These strict controls rule out that the recruitment of EBV-specific CD8⁺ CTL observed in early MS is due to a mere aspecific inflammation-driven process.
- Interestingly, VCA-specific IgG were increased in the CSF of early MS patients as compared to control groups and correlated well with the EBV-specific CD8⁺ T cell response in the CSF. These findings suggest that early MS patients have an increased EBV reactivation in the CSF
- Our data are consistent with a triggering role of EBV in MS.

Anti-VCA IgG are increased in the CSF of early MS patients and correlate with the EBV-specific CD8⁺ CTL response



EBV- and CMV-specific humoral immune responses in the CSF of study patients.

Plasmas and CSF were assayed for anti-VCA IgG, anti-EBNA-1 IgG and anti-CMV IgG. The respective relative antibody indexes (AI), i.e. the recruitment of a given virus-specific antibody in the CSF as compared to the blood, were calculated with the Reber's formula [7]. Horizontal bars represent the median values. NS, non significant, **p<0.01 (Mann-Whitney ranked test), r_s Spearman's non parametric correlation coefficient.

Patients with early MS had an increased relative VCA AI as compared to those with IOND or NIOND. Moreover, when the analyses were performed on the whole cohort, the EBV-specific CD8⁺ CTL response in the CSF significantly correlated with the VCA AI.

REFERENCES

- Ascherio et al. (2007) *Ann Neurol* 61:288
- Haahr et al. (2006) *Rev Med Virol* 16:297
- Giovannoni et al. (2006) *Lancet Neurol* 5:887
- Serafini et al. (2007) *J Exp Med* 204:2899
- Jilek et al. (2008) *Brain* 131:1712
- Jilek et al. (2007) *Clin Immunol* 123:105
- Reiber et al. (1991) *Clin Chem* 37:1153

ACKNOWLEDGEMENTS

RADP is the recipient of grants from the Swiss National Foundation (PP00B-106716) and from the Swiss Society for Multiple Sclerosis.