

Intrathecal Immune Responses to Epstein-Barr Virus in Early Multiple Sclerosis

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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 patients with MS. Interestingly, activated CD8⁺ T cells were located in close contact with these EBV-infected B cells, suggesting that the latter were 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]. We have also reported that there was an enrichment in highly differentiated CD8⁺ T cells in the cerebrospinal fluid (CSF), however we did not address their specificity [6]. Altogether, these data suggest that an abnormal EBV-specific immune response may take place in the central nervous system. Thus, here, we examined the EBV-specific humoral and cellular immune responses in the CSF and blood of patients with early MS or other neurological diseases, separated into inflammatory (OIND) and non-inflammatory (NIND) groups. The neurotropic herpesvirus cytomegalovirus (CMV) served as a control.

MATERIAL AND METHODS:

Patients: We obtained paired blood and CSF samples of 58 patients, including patients who had their first symptoms of MS less than one year prior to our assays (early MS) and patients with OIND and NIND.

Serologies: anti-EBV IgG were measured with a multiplexed immunoassay (Luminex) and anti-CMV IgG with an ELISA in the 58 study patients.

Effector cells: In patients of the cellular immune response arm, PBMC and CSF cells were stimulated with EBV- or CMV-specific pools of immunodominant peptide epitopes known to elicit CD8⁺ T cells, and cultured for 11-14 days in the presence of exogenous IL-2.

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

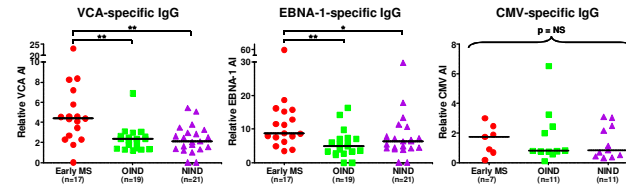
EBER1 detection: In patients of the virological arm, RNA was extracted from CSF cells and reverse-transcribed into cDNA. EBER1 was detected by semi-nested PCR. GAPDH was used as a control for correct RNA isolation.

Clinical and laboratory data of the cohort

	Early MS (n=17)	OIND (n=19)	NIND (n=22)
Age in years ¹	29 \pm 9	44 \pm 17	44 \pm 19
Disease duration in months ¹	2.6 \pm 4.7	1.2 \pm 2.3	0.3 \pm 0.9
CSF leucos per μ l ¹	4 \pm 11	5 \pm 3	1 \pm 0.4
Intrathecal synthesis of IgG	16/17	8/19	0/22
Patients in cellular immune response arm	13/17	15/19	16/22
Patients in virological arm	4/17	4/19	6/22

¹Numbers represent median \pm interquartile range.

Increased Intrathecal VCA- and EBNA-1-specific IgG in Patients with Early MS

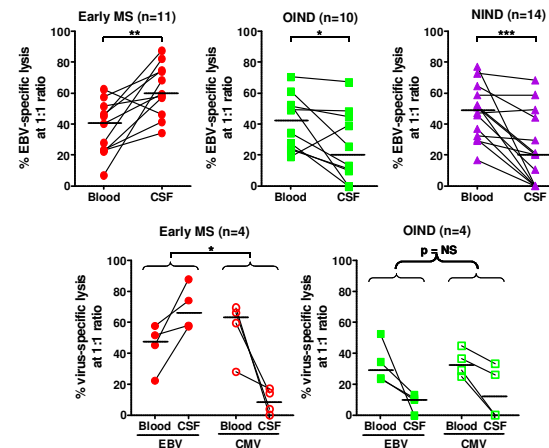


EBV- and CMV-specific humoral immune responses in the CSF of study patients.

Plasma 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 Reiber's formula [7]. Horizontal bars represent the median values. NS, non significant; *p<0.05; **p<0.01 (Kruskal-Wallis and Mann-Whitney ranked tests).

Patients with early MS had an increased relative VCA and EBNA-1 AI as compared to those with OIND and NIND. By contrast, the CMV AI were similar in all the groups of study patients.

High Intrathecal EBV-specific CD8⁺ Cytotoxic T cell Activity in Patients with Early MS



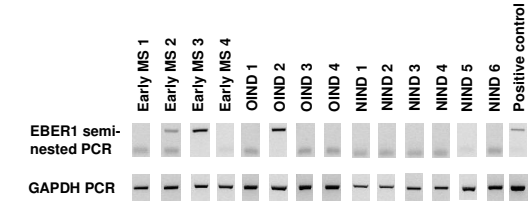
EBV- and CMV-specific CD8⁺ CTL activities in the blood and CSF of study patients.

In peptide pool responsive patients (for EBV: 11/13 early MS, 10/15 OIND and 14/16 NIND; for CMV: 4/9 early MS, 4/8 OIND and 0/1 NIND), 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. Upper panels: *p<0.05, **p<0.01, ***p<0.001 (Wilcoxon ranked test). Lower panels: NS, non significant; *p<0.05 (Fisher's exact test).

There was a significant enrichment in EBV-specific CD8⁺ CTL response in the CSF of patients with early MS. By contrast, this response was significantly lower in the CSF than in the blood of patients with OIND and NIND.

Four early MS and 4 OIND patients i) responded to both EBV and CMV peptide pools and ii) had enough cells in their CSF for the responses against the two viruses to be tested in parallel. In the CSF of the four patients with early MS, there was a recruitment in EBV-, but not CMV-specific CD8⁺ CTL. By contrast, in the four patients with OIND, the CSF response was lower than the blood one for both viruses.

Infrequent Detection of EBER1 Transcripts in CSF Cells



EBV- encoded nuclear RNA1 (EBER1) transcript detection in CSF cells of study patients.

CSF cells were assessed for EBER1 transcript expression by semi-nested PCR. GAPDH was used as a control for correct RNA isolation from CSF cells.

EBER1 transcripts were detected in CSF cells of 2/4 early MS, 1/4 OIND, 0/6 NIND patients. Patients with a positive EBER1 semi-nested PCR had higher CSF cell counts as compared to patients with negative EBER1 semi-nested PCR.

CONCLUSION:

- ▾ Intrathecal humoral immune responses to VCA and EBNA-1 were increased in patients with early MS.
- ▾ In parallel, EBV-specific CD8⁺ CTL were enriched in the CSF of patients with early MS.
- ▾ By contrast, CMV-specific humoral and cellular immune responses were similar in early MS, OIND and NIND patients. Moreover, there was no indication of an increased EBV-specific CD8⁺ CTL activity in the CSF of patients with other neurological diseases, be they inflammatory or not. These strict controls rule out the possibility that the high EBV-specific immune responses observed in the CSF of early MS patients was due to a mere aspecific inflammation-driven process.
- ▾ The increased intrathecal immune responses to EBV suggest that this virus reactivates in the central nervous system of patients with early MS [8, 9]. However, EBER1 transcripts were infrequently observed in the CSF of both early MS and control patients. This may be accounted for by the low frequency of B cells in the CSF.
- ▾ These data strengthen the link between EBV and MS, in particular in the early phase of the disease.

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