Journal of Neuroimmunology 294 (2016) 14–17

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

EBNA1 antigen-specific CD8 + T cells in cerebrospinal fluid of patients with multiple sclerosis

Hebun Erdur [a,*], Veronika Scholz [a], Mathias Streitz [b], Markus Hammer [c], Christian Meisel [b], Constanze Schönemann [d], Klaus-Peter Wandinger [e,f], Berit Rosche [a]

[a] Department of Neurology, Charité—Universitätsmedizin Berlin, Germany
[b] Institute of Medical Immunology, Charité—Universitätsmedizin Berlin, Germany
[c] Department of Nephrology and Internal Intensive Care, Charité—Universitätsmedizin Berlin, Germany
[d] Institute of Transfusion Medicine, Charité—Universitätsmedizin Berlin, Germany
[e] Institute of Clinical Chemistry, University Medical-Centre Schleswig-Holstein, Campus Lübeck, Germany
[f] Department of Neurology, University Medical-Centre Schleswig-Holstein, Campus Lübeck, Germany

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 12 March 2016
Accepted 16 March 2016

Keywords:
Multiple sclerosis
Epstein–Barr virus
Cerebrospinal fluid

ABSTRACT

Epidemiological data suggests that Epstein–Barr virus may be involved in the pathogenesis of Multiple Sclerosis (MS). We aimed to determine the frequency of CD8 + T cells specific for one EBNA1-derived epitope (HPVGEADYFEY) in cerebrospinal fluid (CSF) and blood of patients with MS and other inflammatory neurological diseases (OIND). The frequency of specific CD8 + T cells was assessed by HLA-class-I-binding pentamers restricted to HLA-B35. The frequency of HPVGEADYFEY-specific CD8 + T cells did neither differ significantly in blood nor CSF in MS compared to OIND, but was consistently higher in CSF compared to blood regardless of diagnosis.

1. Introduction

Epidemiological and immunological studies suggest a role of Epstein Barr virus (EBV) in the pathogenesis of Multiple Sclerosis (MS). Individuals with a history of infectious mononucleosis have an increased risk of developing MS (Thacker et al., 2006), while risk of MS in EBV-negative individuals is very low (Levin et al., 2010). Immune control of EBV-infection may be dysregulated in MS patients and thus contribute to autoimmunity (Wandinger et al., 2000). However, the exact mechanisms involved in the interplay of EBV-infection and MS pathogenesis are not well understood (Owens and Bennett, 2012). The presence of an altered frequency of EBNA-1-specific CD8 + T cells in blood and/or CSF of patients with MS compared with other inflammatory neurological diseases (OIND) could indicate an altered immune response to EBV in MS patients. Studies investigating cellular immune responses to EBNA-1 in the CSF of MS patients are scarce, probably due to restriction of the immunogenic EBNA-1-peptide HPVGEADYFEY to HLA-B35. Thus, these analyses can only be conducted in CSF of patients with the HLA haplotype HLA-B35, which is expressed in only approximately 10% of the general population (Müller et al., 2003). Furthermore, the detection of virus-specific CD8 + T cells in CSF requires a rapid analysis of CSF probes after lumbar puncture and is also impaired by the low number of cells in CSF.

We aimed (i) to determine the frequency of CD8 + T cells specific for one EBNA1-derived epitope (HPVGEADYFEY) in blood and CSF of patients with MS. For the purpose of comparison, (ii) we assessed frequencies of CD8 + T cells specific for one CMV-derived epitope (NLVPMVATV of pp65) in blood and CSF, and (iii) corresponding frequencies of HPVGEADYFEY- and NLVPMVATV-specific CD8 + T cells in patients with OIND.

2. Methods

2.1. Patients

Patients were recruited from the Department of Neurology, Charité—Universitätsmedizin Berlin. Diagnosis of CIS, MS (according to the 2005 McDonald criteria) (Polman et al., 2005), and OIND was made by the attending neurologist. Routine CSF analysis was conducted for diagnostic purposes and included cell count, IgG, IgM, IgA serum/CSF-quotient and oligoclonal bands. Analysis of CSF in MS patients was performed before initiation of therapy with corticosteroids. EBV and CMV IgG and IgM antibody status in serum was determined by ELISA. As diagnostic work-up was not to be delayed by HLA-class

Abbreviations: MS, multiple sclerosis; CSF, cerebrospinal fluid; CMV, cytomegalovirus; EBV, Epstein Barr virus; EBNA1, EBV-encoded nuclear antigen-1.

* Corresponding author at: Department of Neurology, Campus Benjamin Franklin, Charité—Universitätsmedizin Berlin, Hindenburgdamm 30, 12200 Berlin, Germany.
E-mail address: hebun.erdur@charite.de (H. Erdur).

http://dx.doi.org/10.1016/j.jneuroim.2016.03.010
0165-5728/

testing, staining of CSF and HLA-testing were done simultaneously. Because of low cell numbers in CSF, simultaneous staining for specific CD8 + T cells for EBV and CMV was not conducted in all patients. Furthermore, due to logistic reasons, pentamer staining was not performed in all samples. The procedure of staining of CSF and whole blood is described in the supplemental material. Only patients with HLA-A02 or HLA-B35 genotype, positive CMV and/or positive EBV serology were eligible for analysis. The study was approved by the local ethics committee and subjects or representatives provided informed consent.

2.2. Statistical analysis

Results of FACS staining were obtained using CellQuest software. These were statistically analysed by means of an independent two-sided t test. Confidence intervals for proportions were calculated using the Wilson score method without continuity correction (Newcombe, 1998). p values < 0.05 were considered significant. SPSS 19 was used for all statistics.

3. Results

3.1. Baseline characteristics

A total of 96 patients were screened. An inflammatory neurological disease was diagnosed in 81 patients (of these, CIS was diagnosed in 18 patients, RR-MS in 19, and SP-MS and PP-MS each in one case; 24 patients had a viral inflammatory disease, nine a bacterial and nine patients other inflammatory diseases). In 15 patients, a non-inflammatory neurological disease was diagnosed.

HPVGEADYFEY-specific CD8 + T cells were detected in 11 patients (five with RR-MS, five with viral or bacterial CNS-inflammation, and one patient with transient diplopia and signs of inflammation in CSF). NLVPMVATV-specific CD8 + T cells were present in nine patients (two with CIS, two RR-MS, four with viral or bacterial CNS-inflammation and one patient with primary angiitis of the CNS). In patients with viral inflammatory CNS-disease, herpes simplex virus (types 1 and 2) and varicella zoster virus were the main causes.

Table 1 lists all patients with positive staining for HPVGEADYFEY- and NLVPMVATV-specific CD8 + T cells and the respective proportions of all CD8 + T cells. Causes of inflammatory disease are also listed in Table 1. Fig. 1 shows the HPVGEADYFEY- and NLVPMVATV-specific CD8 + T cells in blood and CSF of a patient with RR-MS and a patient with viral meningitis, respectively.

Table 1
Overview of antigen-specific CD8 + T cells as % of all CD8 + T cells.

Patient	Diagnosis	Frequency of specific CD8 + T cells in blood in %	Frequency of specific CD8 + T cells in CSF in %
HPVGEADYFEY(EBNA1)-specific CD8 + T cells			
1	RR-MS	0.71	1.81
2	RR-MS	0.4	3.83
3	RR-MS	1.02	3.39
4	RR-MS	0.28	ND
5	RR-MS	0.06	ND
	MS patients (mean)	0.49% ± 0.38%	3.01% ± 1.06%
6	Viral meningitis (HSV type 1)	0.34	3.01
7	Viral meningitis (HSV type 2)	0.09	0.12
8	Ramsay Hunt syndrome	0.42	1.77
9	Bacterial meningitis	0.2	2.3
10	Bacterial meningitis	0.13	1.92
11	Transient neurological disorder with signs of inflammation in CNS	0.13	0.44
	OIND patients (mean)	0.22% ± 0.13%	1.59% ± 1.11%
Statistics (t-test)	MS vs. OIND patients	p = 0.18	p = 0.11
NLVPMVATV(pp65 of CMV)-specific CD8 + T cells			
12	CIS	3.62	1.56
13	CIS	0.33	ND
4	RR-MS	0.51	ND
14	RR-MS	0.96	ND
	CIS/MS patients (mean)	1.36% ± 1.53%	
15	Viral meningitis (HSV type 2)	4.93	1.99
16	Herpes zoster	ND	2.1
17	Ramsay Hunt syndrome	4.52	0.54
18	Bacterial meningitis	ND	0.19
19	Primary angiitis of the CNS	1.63	0.73
	OIND patients (mean)	3.69% ± 1.80%	
Statistics (t-test)	MS vs. OIND patients	p = 0.12	

3.2. Frequency of HPVGEADYFEY- and NLVPMVATV-specific CD8 + T cells in blood and CSF

The frequency of HPVGEADYFEY-specific CD8 + T cells in blood as percent of all CD8 + T cells was not significantly higher in MS patients compared to OIND (MS: mean 0.49% ± 0.38% vs. OIND: mean 0.22% ± 0.13%; p = 0.18). Also, the proportion of NLVPMVATV-specific CD8 + T cells in blood did not differ significantly between CIS/MS and OIND (CIS/MS: mean 1.36% ± 1.53% vs. OIND: mean 3.69% ± 1.80%; p = 0.12). When frequency in CSF was analysed, proportion of HPVGEADYFEY-specific CD8 + T cells did not differ significantly in both patient groups (MS: mean 3.01% ± 1.06% vs. OIND: mean 1.59% ± 1.11%; p = 0.11).

3.3. Comparison of distribution of HPVGEADYFEY- and NLVPMVATV-specific CD8 + T cells

In all patients (9/9, 95% CI 0.70–1.0) with simultaneous pentamer-staining in blood and CSF, HPVGEADYFEY-specific CD8 + T cells were more frequent in CSF than in blood. On the contrary, NLVPMVATV-specific CD8 + T cells were more frequent in blood compared to CSF in all patients (4/4, 95% CI 0.51–1.0) with simultaneous pentamer-staining in blood and CSF.

4. Discussion

Our study revealed several novel findings. First, we were able to detect directly CD8 + T cells specific for one EBNA1-derived epitope (HPVGEADYFEY) in the CSF of patients with MS and OIND by staining with HLA-matched pentamers. Second, we could show that HPVGEADYFEY-specific CD8 + T cells were more frequent in CSF compared to blood regardless of diagnosis. Third, for CMV-specific cells (CD8 + T cells specific to NLVPMVATV of pp65), the opposite was true with a higher frequency in blood compared to CSF. Of note, this observation of a different distribution pattern was present in every patient with simultaneous staining of blood and CSF.

Several studies have investigated specific immune responses of CD8 + T cells after stimulation with EBV-peptides. Höllsberg et al. have shown a selective increased cellular response in MS patients upon stimulation with two peptides restricted to HLA-A2 and HLA-B7 (Höllsberg et al., 2003), whereas Gronen et al. could not confirm an increased response of CD8 + T cells against the HLA-B7 restricted peptide RPPIFIRRL of EBNA-3A (Gronen et al., 2006). Lünemann et al. showed an increased cellular response of CD4 + T-cells to peptides from the C-terminal region of EBNA-1, but this increase was not significant in CD8 + T cells (Lünemann et al., 2006). In another study, Lünemann et al. showed an increased frequency of IFN-gamma producing T-cells (irrespective of CD4 or CD8 status) in patients with CIS after stimulation with EBNA-1, but this increase was not present after stimulation with HLA-class-I restricted peptides from EBNA3a, 3b, 3c, BZLF1, BRLF1, and BMLF1 (Lünemann et al., 2010). Regarding CD8 + T cells in CSF, Jaquiéry et al. could show an enrichment of EBV-specific CD8 + T cells in patients with early MS, while there was no enrichment of CMV-specific CD8 + T