

Characterization of viral-specific CD8+ T cells in multiple sclerosis patients

Samantha Jilek¹, Andreas Lysandropoulos², Anastasia Zekeridou², Mathieu Canales¹, Myriam Schluemp², Giuseppe Pantaleo¹, Renaud Du Pasquier^{1,2}



Service of Immunology and Allergy, Department of Medicine¹ and Service of Neurology, Department of Clinical Neurosciences², CHUV, Lausanne, Switzerland

ABSTRACT:

Introduction: Epstein-Barr virus (EBV), in contrast to cytomegalovirus (CMV), has been repeatedly associated with multiple sclerosis (MS). We have previously shown that there is a high peripheral as well as intrathecal activation of CD8+ T cell against EBV, but not CMV, early in the course of MS. However, the trigger of this increased EBV-specific CD8+ T cell response, IFN- γ -mediated, remains obscure. It could result from a higher EBV viral load. Alternatively, it could be due to intrinsically deficient EBV-specific T cell responses, cytotoxic granules-mediated, such as it has been demonstrated in various mouse viral infection. Thus, we performed an in-depth study of the phenotype of ex vivo EBV- and CMV-specific CD8+ T cells in MS patients and control patients.

Methods: We analysed the profile of cytotoxic granules, including perforin and granzymes, in EBV- and CMV-specific CD8+ T cells in a cohort of 17 early MS patients, 20 late MS, 30 other neurological diseases (OND) patients and 13 healthy control subjects. Ex vivo analysis of EBV- or CMV-specific CD8+ T cells was performed with peptide-tetramer complexes coupled to CCR7 and CD57 markers in conjunction with perforin, granzymes A, B and K staining. Cytotoxic activity of EBV-specific CD8+ T cells was assessed with a function CTL CFSE assay.

Results: Using different HLA class I tetramers for EBV (A*0201, B*0702, B*0801) and CMV (A*0201, B*0702), we found that the frequency of EBV- or CMV-specific CD8+ T cells were similar in all study subjects. Most of EBV- and CMV-specific CD8+ T cells were highly differentiated (CCR7-) and a various proportion expressed the exhaustion marker CD57. Finally, the cytotoxic profiles (perforin and granzymes) of MS patients were very similar to OND or HC subjects, be it EBV- or CMV-specific CD8+ T cells.

Conclusions: EBV-specific CD8+ T cells are increased in early MS, however their cytotoxic profile is not impaired, suggesting that the CD8+ T cell response in MS patients is not intrinsically impaired. However, the cytotoxic activity of CTL decreased in late MS patients suggesting an exhaustion of EBV-specific CD8+ T cells possibly due to EBV reactivation.

INTRODUCTION:

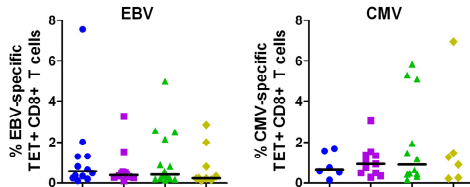
Epstein-Barr virus (EBV), in contrast to cytomegalovirus (CMV), has repeatedly been associated with multiple sclerosis (MS) [1-5]. However, if EBV is associated with MS, is it really a trigger of the disease or would it rather be an accompanying marker associated with the degree of activity of MS? We demonstrated previously that early in the course of MS, high peripheral as well as intrathecal activation of CD8+ T cells against EBV, but not CMV, early in the course of the disease [5-6], thus strengthening the link between EBV and MS. However, the trigger of this high EBV-specific CD8+ T cell response is unknown, and could be either an increased EBV viral load or an intrinsically deficient EBV-specific T cell response. To test this, we performed an in-depth study of ex vivo EBV- and CMV-specific tetramer-positive CD8+ T cells in MS patients and control patients, both at the phenotypic (cytotoxic granules) and functional level (cytotoxicity).

Cytotoxic activity of EBV-specific CD8+ T cells seems to be impaired in MS patients with longer disease duration

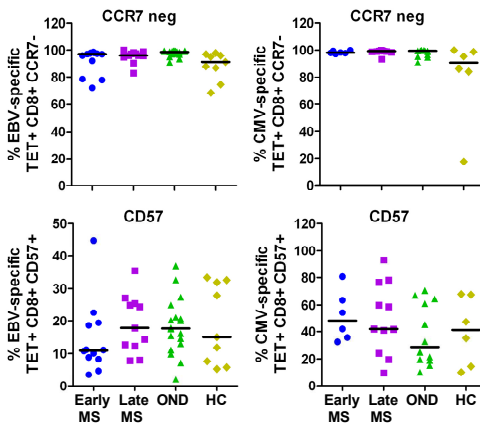
Cytotoxic activity of EBV-specific tetramer-positive CD8+ T cells. Cytotoxic activity of EBV-specific tetramer-positive CD8+ T cells seems to be impaired in patients with late MS, although this result was not significant. This impairment was rescued when we added IL-2 to the culture, implying that there might be either an in vivo deficit in IL-2 or a suboptimal response to physiological concentrations of IL-2. The magnitude of the cytotoxic response was correlated with the interval between the first symptom indicative of MS and our assays.

EBV- and CMV-specific tetramer-positive CD8+ T cells are highly differentiated, and have a similar profile of cytotoxic granules in MS patients and controls

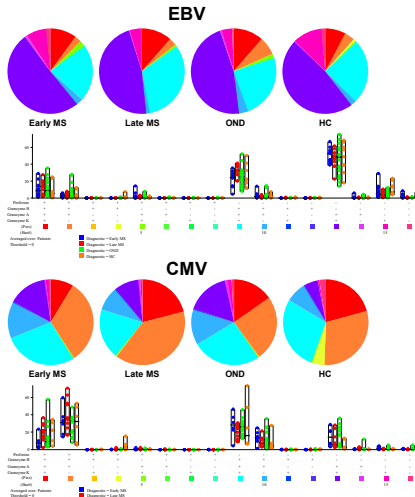
Frequency of EBV- and CMV-specific tetramer-positive CD8+ T cells. There was no difference in the frequency of tetramer positive cells in early or late MS patients versus control subjects (OND or HC; upper panels). In addition, A2-, B7- or B8-restricted tetramer-positive CD8+ T cells were similar in all subjects.



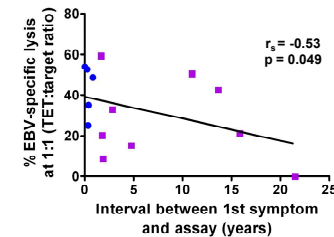
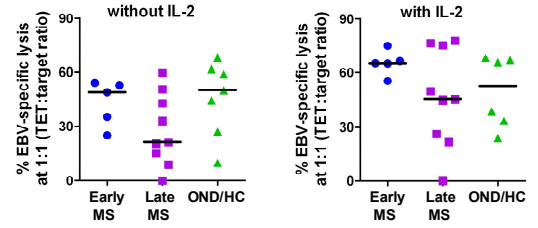
Surface marker expression on viral-specific tetramer-positive CD8+ T cells. Viral-specific CD8+ T cells were mainly CCR7-, indicative of a more differentiated phenotype (middle panels), and about a third of them harbour the marker of exhaustion (CD57; lower panels). Again, no difference was found between the MS patients (early or late) and the control groups, as well as between A2-, B7- or B8-restricted tetramer-positive CD8+ T cells.



Profiles of cytotoxic granules in viral-specific tetramer-positive CD8+ T cells. Such as previously described [7], EBV and CMV had each a distinct signature in terms of cytotoxic granule composition. However, there was no difference between the different groups. In addition, phenotype of EBV-specific A2-, B7- and B8-restricted tetramer-positive CD8+ T cells or CMV-specific A2- and B7-restricted tetramer positive CD8+ T cells were comparable. This suggests that EBV-specific CD8+ T cells are not impaired in MS patients.



We tested 261 subjects for presence of EBV- and CMV-positive tetramers (A*0201, B*0702 and B*0801; A*0201 and B*0702, respectively). Of these cohort, 85 were characterized in more details for their cytotoxic granule phenotype (50 for EBV and 35 for CMV). Cells were stained as previously described [7] using the following markers: TET, CD8, CCR7, CD57, perforin, granzyme A, granzyme B, granzyme K. Data were acquired on a LSR II and analysed with FlowJo and SPICE. Each dot represented a patient. Early MS are defined as patients who had their first symptom indicative of MS less than one year before tetramer staining. MS, multiple sclerosis; OND, other neurological diseases; HC, healthy controls; TET, tetramer. * < 0.05 (two-tailed Student t test).



Fifteen subjects positive for EBV-specific B7 tetramer were used in functional CFSE CTL assays [6]. Bulk PBMC were stimulated with HLA B*0702 EBV peptide for 7 days in the presence or absence of IL-2 (20 U/ml) in RPMI/8% at day 7, the effector cells were stained with the EBV-specific B7 tetramer and the number of effectors for the functional CFSE CTL assays normalized in terms of tetramer-positive effectors. Final effector TET+ target ratios were the following: 0:1 / 0.1:1 / 0.25:1 / 0.5:1 / 1:1 / 2:1. Functional CFSE CTL assays were performed as previously described [6]. MS, multiple sclerosis; OND, other neurological diseases; HC, healthy controls; TET, tetramer.

CONCLUSION:

- EBV- and CMV-specific CD8+ T cells are a highly differentiated (CCR7-) but not exhausted (CD57-) subset. Interestingly enough, there was no difference in the frequency of A2-, B7- or B8-restricted tetramer-positive CD8+ T cells for both viruses.
- Cytotoxic granules distribution is different between EBV- and CMV-specific CD8+ T cells, such as reported previously [7]. However, no difference between MS patients and controls (OND and HC) was found.
- In contrast, differences in the functional capacity of EBV-specific CD8+ T cells appeared in MS patients. Indeed, patients with longer disease duration tend to have lower functional cytotoxicity as compared to early MS patients or controls.
- Impairment in cytotoxic activity of EBV-specific CD8+ T cells was rescued with IL-2, suggesting that there might be either an in vivo deficit in IL-2 or a suboptimal response to physiological concentrations of IL-2.
- In conclusion, phenotypes of EBV-specific CD8+ T cells in MS patients are not different from controls, however preliminary data on their functional capacity demonstrate that EBV-specific CD8+ T cell cytotoxicity is affected by a longer disease duration. Although MS patients do not have a deficit in CD8+ cytotoxic lymphocytes, our data suggest that continuous restimulation of EBV-specific CD8+ T cells in MS patients either due to a higher EBV viral load or to a deficit in the control of EBV replication, might lead to exhaustion of these cells as seen by decreased IFN- γ secretion [5] and loss of EBV-specific cytotoxicity.

REFERENCES:

- Aschero et al. (2007) Ann Neurol 61:288
- Pohl et al. (2006) Neurology 67:2063
- Alotaibi et al. (2004) JAMA 291:1875
- Levin et al. (2005) JAMA 293:2496
- Jilek et al. (2008) Brain 131:171
- Jaquifry et al. (2010) EJI 40:878
- Harari et al. (2009) J Virol 83:2862

ACKNOWLEDGEMENTS:

We thank Kim Elifsen Lavio, Emmanuelle Medjtina and the NIH Tetramer Core Facility for providing the EBV and CMV tetramers. This work is supported by the Swiss National Foundation, the Swiss Society for Multiple sclerosis and the Bragi foundation.