Cytotoxic CD8 T cells (the effectors) against EBV-infected B cells (the targets): clues for virus-driven immunopathology in multiple sclerosis

Barbara SERAFINI, Barbara ROSICARELLI, Caterina VERONI, Francesca ALOISI

Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy

INTRODUCTION

Epstein-Barr virus (EBV) is strongly associated with MS but it is still unclear how EBV infection connects to CNS pathology. Altogether, increased anti-EBV immune reactivity in MS patients (1), the B-cell tropism of EBV (2) and the efficacy of B-cell depleting therapy in MS (3) raise the possibility that EBV-infected B cells and anti-EBV immunity are instrumental in boosting an immunopathological response that harms the CNS. Consistent with this hypothesis are the following findings: predominance of cytotoxic CD8 T cells, which have a key role in the control of viral infections, in the MS brain (4); selective enrichment of EBV-specific CD8 T cells in patient CSF (5,6); alterations in frequency and function of EBV-specific CD8 T cells in patient blood (1). Moreover, our studies in postmortem MS brain tissue show presence of an active EBV infection in CNS-infiltrating B-lineage cells (7,8) [recently confirmed in an independent study (9)], CD8 T cell-mediated cytotoxicity toward EBV-infected cells (7,8) and an association between EBV reactivation and extent of CNS inflammation (7,10,11).

Aiming at identifying the immune "culprits" responsible for a detrimental antiviral response within the CNS, we stained postmortem brain tissue donated by 12 persons with MS (all brain samples were obtained from the UK MS Society Tissue Bank) with HLA class I pentamers (ProImmune Pro5® MHC Class I pentamers) to: i) identify CNS-infiltrating EBV-specific CD8 T cells; ii) compare the frequency of EBV-specific CD8 T cells with that of CD8 T cells recognizing other common viruses or a putative myelin autoantigen; iii) study the cytotoxic effector function of CNS-infiltrating EBV-specific CD8 T cells and their spatial proximity to virus infected B lineage cells. Because NK cells are essential in the control of EBV lytic infection (12), we also searched for NK cells in active WM lesions and meningeal B-cell follicles, where EBV was found to reactivate in plasma cells (7).

RESULTS

Table 1 HLA class I allele restriction of MS brain tissue donors and antigens and peptide epitopes analyzed by *in situ* pentamer staining

MS donor ID	MS donor HLA class I alleles suitable for in situ pentamer binding	EBV antigen/ epitope coordinates	EBV epitope sequence	hCMV antigen/ epitope coordinates	hCMV epitope sequence	Influenza A antigen/epitope coordinates	Influenza A epitope sequence	MBP/ epitope coordinates	MBP epitope sequence
MS79 MS286 MS 289 MS 342	A*0201	EBNA-3C /284-293	LLDFVRFMGV	pp65 495-504	NLVPMVATV	MP 58-66	GILGFVFTL	MBP 110-118	SLSRFSW GA
		LMP-1 / 125-133	YLLEMLWRL						
		LMP-2 / 356-364	FLYALALLL						
		LMP-2 / 426-434	CLGGLLTMV						
		BRLF1 / 109-117	YVLDHLIVV						
		BMLF-1 / 259-267	GLCTLVAML						
		BALF-4 / 276–284	FLDKGTYTL						
MS330 MS356 MS289 MS342	B*0702	EBNA-3A /247-255	RPPIFIRRL	pp65 265-275	RPHERNGFTVL	NP 473-481	SPIVPSFDM		
		EBNA-3C/ 881-889	QPRAPIRPI						
		BMRF1/116-128	RPQQGGSRPEFVKL						
MS92 MS121 MS154 MS180 MS234 MS352	B*0801	EBNA-3A /193-201	FLRGRAYGL	IE1 88-96	QIKVRVDMV				
		BZLF-1/190-197	RAKFKQLL						

Fig 1 HLA class I pentamers coupled with EBV-protein derived peptides bind to CD8+ cells in brain sections from HLA-matched MS donors

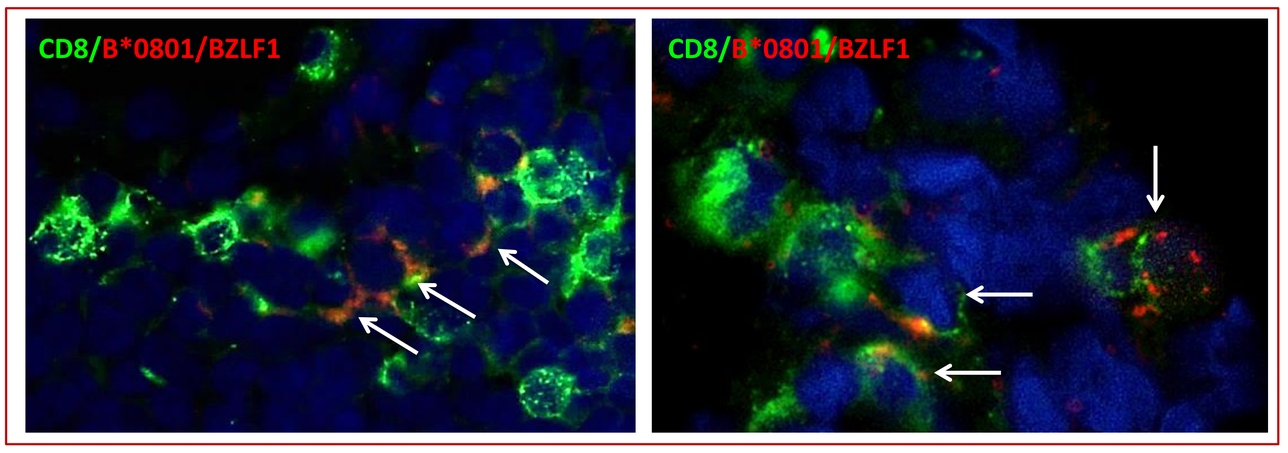
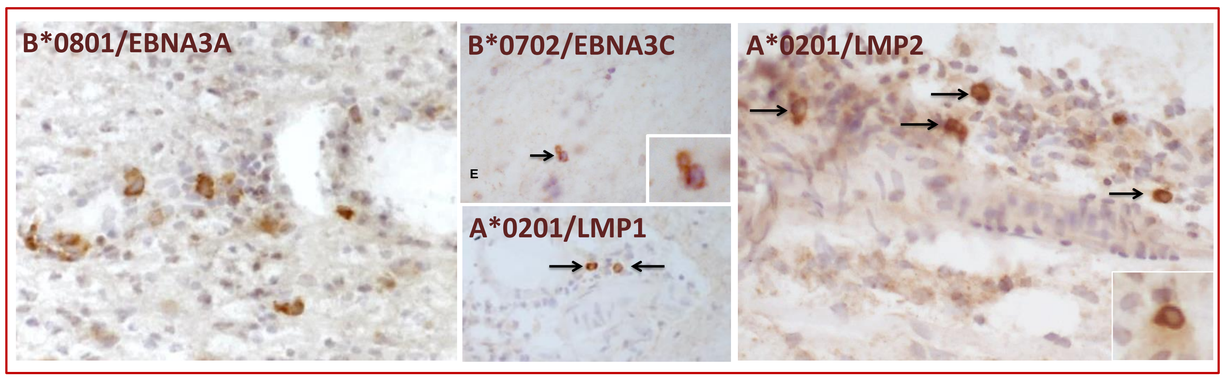


Fig 3 EBV lytic Ag-specific CD8 T cells are visualized in WM lesions and meninges (11/12 MS cases)

Fig 2 EBV latent Ag-specific CD8 T cells are visualized in WM lesions and meninges (11/12 MS cases)



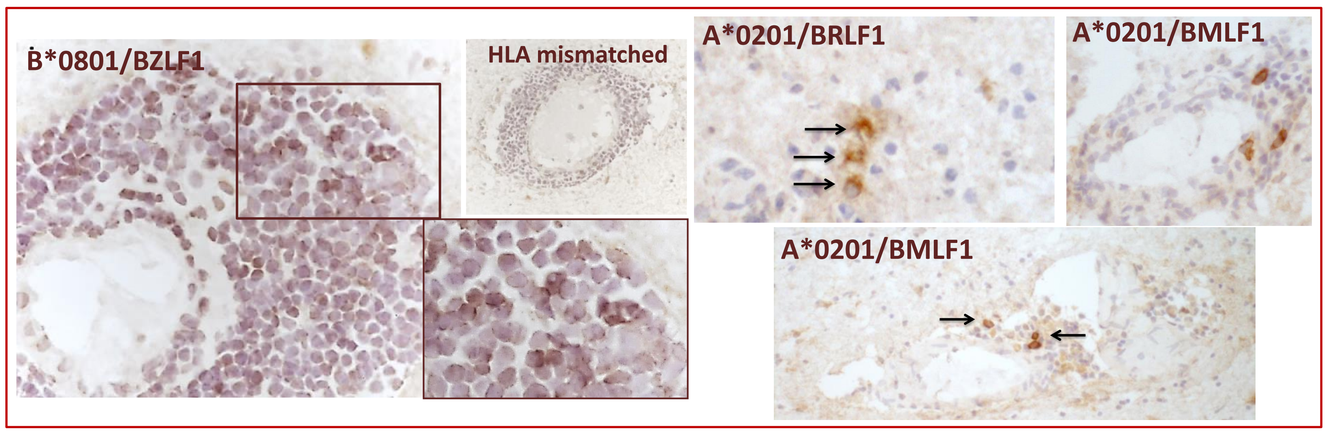


Fig 4 EBV-specific CD8 T cells preferentially accumulate in the MS brain

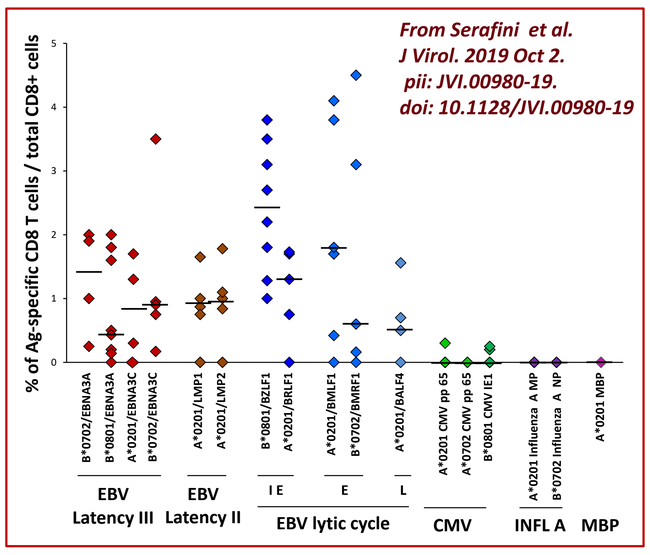


Fig 5 CNS-infiltrating EBV-specific CD8 T cells are cytotoxic and contact EBV infected cells

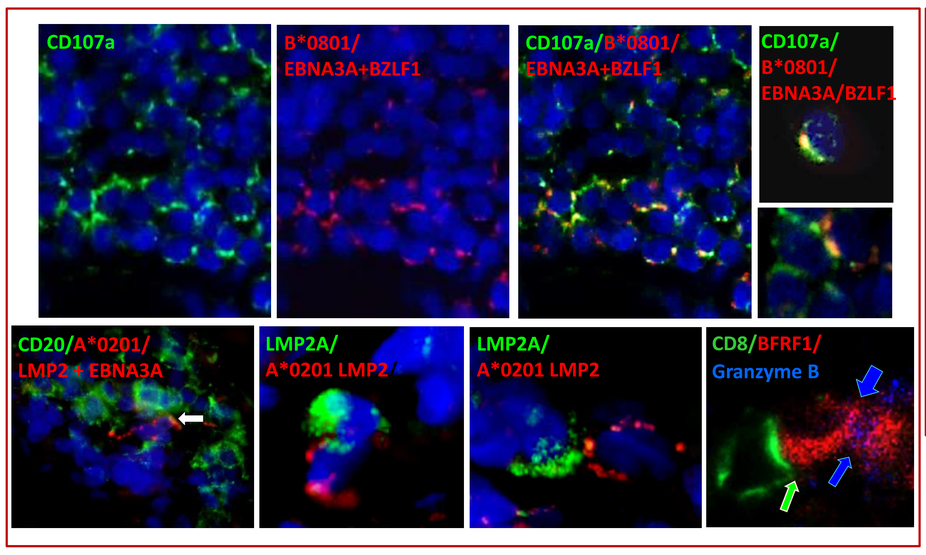
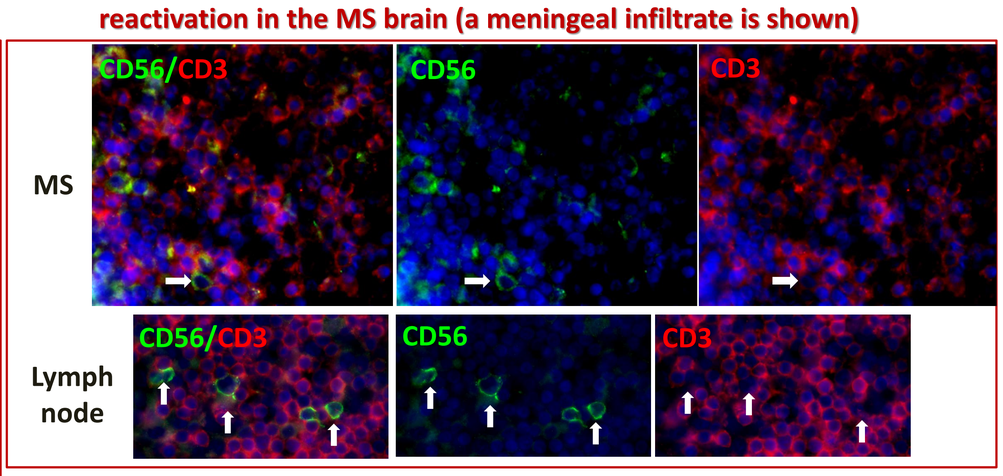


Fig 6 CD56+ CD3- NK cells are very rarely detected at sites of EBV reactivation in the MS brain (a meningeal infiltrate is shown)



DISCUSSION

The key finding of this study is that EBV-specific CD8 T cells are commonly found in the MS brain and are more frequent than CMV-specific CD8 T cells. CD8 T cells recognizing influenza A virus or MBP are not detected. These data suggest that migration of EBV-specific CD8 T cells in the MS brain results from active recruitment rather than non specific extravasation due to the local inflammatory process.

EBV antigen recognition by CNS-infiltrating CD8 T cells encompasses a wide range of proteins expressed in different phases of the EBV life cycle. Furthermore, EBV-specific CD8 T cells recruited to the MS brain have a cytotoxic phenotype and contact EBV infected cells. These results suggest that CNS-infiltrating EBV-specific CD8 T cells become activated after recognition of their cognate antigen on infected cells and may kill their target cells.Probably due to EBV capability of evading immune surveillance, the cytotoxic response fails to fully control intracerebral EBV infection and goes awry causing neural cell damage.

The selective enrichment of EBV-specific CD8 T cells in postmortem MS brain (this study) and in the CSF of MS patients (5,6) supports a pathogenic model of MS where anti-EBV immunity causes inflammation in the CNS. Failure to eradicate a chronic active EBV infection in the MS brain should lead to a vicious circle of viral antigens stimulating the anti-EBV immune response which maintains local inflammation. In this respect, MS shows analogies with HTLV-1-associated myelopathy/HAM, an infrequent neurological complication of HTLV-1 infection. In HAM, circulating HTLV-1-infected T cells invade the CNS and trigger a cytotoxic immune response against the virus which inadvertently damages neural cells (Box 1).

An EBV-centered pathogenic model of MS may explain why B-cell depleting therapy, which is used in EBV-associated lymphoproliferative diseases/lymphomas to eliminate EBV transformed B cells, is highly effective in MS. If EBV is the main antigenic stimulus promoting immune-mediated CNS pathology in MS, it should be possible to treat MS by normalizing the EBV-host balance with antiviral drugs (13) or immunotherapy (14).

This study was funded by Fondazione Italiana Sclerosi Multipla (grant 2016/R/27 to BS) and Italian Ministry of Health (grant RF-2011-02347228 to FA)

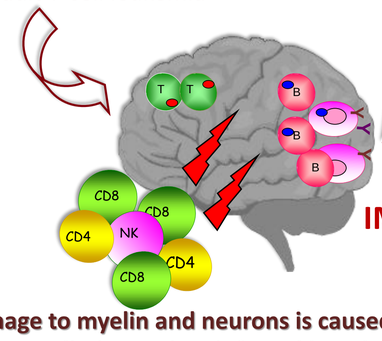
Box 1 Two inflammatory CNS diseases, two different viruses infecting and activating lymphocytes, a common mechanism driving CNS pathology?

HTLV-1-ASSOCIATED MYELOPATHY/ TROPICAL SPASTIC PARAPARESIS

- Inflammatory/demyelinating disease of the CNS.
- HTLV-1/RNA virus
- Infects and persists in T cells
- Promotes T-cell activation/proliferation
- Causes HAM in <2% of infected individuals
- Oncogenic: Adult T-cell leukemia

MULTIPLE SCLEROSIS

- Inflammatory/demyelinating disease of the CNS
- EBV/DNA virus
- Infects and persists in B cells
- Promotes B-cell activation/ proliferation
- Causes disease in 1-2 in 1000 infected individuals
- Oncogenic: LPD, Lymphomas, nasopharingeal carcinoma
- Infectious mononucleosis



VIRUS-INDUCED IMMUNOPATHOLOGY

Collateral damage to myelin and neurons is caused by the immune system attempt to eliminate virus infected lymphocytes in the CNS

REFERENCES

1. Lucas RM et al. *J Neurol Neurosurg Psychiatry* 2011;82:1142-48
2. Thorley-Lawson DA. *Curr Top Microbiol Immunol* 2015;390:151-209
3. Greenfield AL, Hauser SL. *Ann Neurol* 2018;83:13-26
4. Denic et al. *Expert Opin Ther Targets* 2013;17:1053-66
5. Jaquiéry E et al. *Eur J Immunol* 2010;40:878-87
6. van Nierop GP et al. *Mult Scler* 2016;22:279-91
7. Serafini B et al. *J Exp Med* 2007;204:2899-912
8. Veroni C et al. *J Neuroinflammation* 2018;15(1):18.
9. Moreno MA et al. *Neurol Neuroimmunol Neuroinflamm* 2018;5(4):e466
10. Serafini B et al. *J Neuroimmunol* 2017;307:14-17
11. Serafini B et al. *J* Neuroimmunol 2018;319:9-12
12. Münz C. *Crit Rev Immunol* 2014;34:501-7
13. Lycke J. *Acta Neurol Scand* 2017;136:45-8
14. Pender MP et al. *JCI Insight* 2018;3(22). pii:124714.