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

Emilie Jaquiéry1, Samantha Jilek Terrasse1, Myriram Schluemp2, Pascal Meylan3, Andreas Lysandropoulos2, Giuseppe Pantaleo1, Renaud Du Pasquier1,2

Services of 1Immunology and Allergy and 2Neurology, 3Institute of Microbiology, CHUV, Lausanne

INTRODUCTION:

Epstein-Barr virus (EBV) has been consistently associated with multiple sclerosis (MS) by examining cellular and humoral immune responses in the blood and CSF. Our group has shown that EBV-specific cytotoxic T lymphocyte (CTL) activity against EBV-infected B cells [4]. In addition to raising the question of the involvement of EBV in MS, these data point toward a pathogenic role of CD8+ T cells in this disease. Linking CD8+ T cells and EBV, we have located in neo-follicles in the meninges have been detected in patients with MS, but not with other inflammatory neurological diseases. Activated CD8+ T cells present in the vicinity of these neo-follicles were suggestive of ongoing cytotoxic activity against EBV-infected B cells [4]. In addition to raising the question of the involvement of EBV in MS, these data point toward a pathogenic role of CD8+ T cells in this disease. Linking CD8+ T cells and EBV, we have located in neo-follicles in the meninges have been detected in patients with MS, but not with other inflammatory neurological diseases. Activated CD8+ T cells present in the vicinity of these neo-follicles were suggestive of ongoing cytotoxic activity against EBV-infected B cells [4]. In addition to raising the question of the involvement of EBV in MS, these data point toward a pathogenic role of CD8+ T cells in this disease. Linking CD8+ T cells and EBV, we have located in neo-follicles in the meninges have been detected in patients with MS, but not with other inflammatory neurological diseases. Activated CD8+ T cells present in the vicinity of these neo-follicles were suggestive of ongoing cytotoxic activity against EBV-infected B cells [4].

MATERIAL AND METHODS:

Patients obtained paired blood and CSF samples of 123 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 IgGs were measured with a multiplexed immunosay (lumien) and anti-CMV IgGs with an ELSA in the 123 study patients. Effectors: in patients of the cellular immune response arm, PBMC and CSF cells were stimulated with EBV- or CMV-specific pools of immunodominant peptide pools known to elicit CD8+ T cells, and cultured for 11-14 days in the presence of exogenous IL-2. Functional CFSE cytotoxic T lymphocyte (CTL) assay: Target cells were prepared by staining autologous PBMC with CFSE and loading them with EBV- or CMV peptides. After 18 h of incubation with increasing ratios of effector cells, surviving target cells were quantified by flow cytometry. Tetramer staining: Effector cells from the blood and CSF of a subset of patients were stained with the HLA-A*0201/BMLF-1 tetramer. BMLF-1 is a protein which is part of the lytic cycle of EBV, while EBNA-3A is a latent protein of EBV.

Intrathecal CMV-specific Immune Responses Are Similar in All Categories of Patients

Early MS patient 1

NIND patient 1

PBMC

EBV

CMV

Early MS patient 2

NIND patient 3

PBMC

EBV

CMV

Early MS patient 3

NIND patient 4

PBMC

EBV

CMV

PBMC

EBV

CMV

CMV tetramer staining in blood and CSF effector cells of study patients. CSF and blood effector cells were stained with the HLA-A*0201/BMLF-1 tetramers and IFN-γ, intracellular cytokine staining in three HLA-A*0201+ study patients (respectively) and IFN-γ, intracellular cytokine staining in three HLA-A*0201+ study patients (respectively). PBMC and CSF effector cells generated upon stimulation with EBV peptide pool and cultured for 11 to 14 days contained tetramer-positive cells, confirming that our pool of EBV peptides indeed led to the clonal expansion of epitope-specific CD8+ CTL. Interestingly, these tetramer data are consistent with the functional (IFN-γ) assay, since there was enrichment in tetramer-positive cells in the CSF of the two early MS, but in none of the four NIND patients.

CONCLUSION:

- Intrathecal 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.

These data strengthen the link between EBV and MS, in particular in the early phase of the disease.