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JC virus induces a vigorous CD8$^+$ cytotoxic T cell response in multiple sclerosis patients

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Abstract

We sought to compare the ongoing CD8$^+$ cytotoxic T lymphocytes (CTL) immune response of MS patients to self and viral antigens. Using ^{51}Cr release and tetramer staining assays, we found that the CTL response against VP1, the major capsid protein of the polyomavirus JC (JCV), was significantly higher than the one against epitopes of MBP and PLP. The JCV-specific CTL response was also significantly stronger in MS patients than healthy control subjects. These findings may shed a new light on the recent events related to the development of progressive multifocal leukoencephalopathy in three natalizumab-treated patients.

Keywords: Multiple sclerosis; Myelin; Progressive multifocal leukoencephalopathy; JC virus; Natalizumab; CD8$^+$T cells

1. Introduction

The etiology of multiple sclerosis (MS) remains elusive. It has been hypothesized that both genetic and environmental factors, including viral infections, contribute to the manifestation and progression of the disease. Although CD4$^+$T lymphocytes have been classically implicated in causing MS, CD8$^+$T lymphocytes appear to be involved in the pathogenesis of this disease as well. Indeed, recent data from two animal models of MS, experimental autoimmune encephalomyelitis (EAE) (Huseby et al., 2001; Sun et al., 2001) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (Johnson et al., 2001; Rivera-Quinones et al., 1998; Tsunoda et al., 2002), suggest that CD8$^+$T cells are involved in demyelination and axonal damage. CD8$^+$T cells recognize an antigen presented in association with a major histocompatibility complex (MHC) class I molecule, whereas CD4$^+$T cells have an affinity for an antigen presented by a MHC class II molecule. In the CNS of MS patients, MHC class I molecules are expressed at a higher level than MHC class II molecules (Medana et al., 2001) and CD8$^+$T cells are more numerous than CD4$^+$T cells in MS lesions, particularly at the early stage of the disease (Gay et al., 1997). Moreover, CD8$^+$T cells in the cerebrospinal fluid (CSF) and in the brain lesions of MS patients have a monoclonal or oligoclonal distribution, suggesting that they proliferate in reaction to antigen(s) present within the CNS (Babbe et al., 2000; Jacobsen et al., 2002; Skulina et al., 2004). Altogether, these data point toward a potential role of CD8$^+$T cells in the pathogenesis of MS.

In this study, we sought to compare the ongoing CD8$^+$cytotoxic T lymphocytes (CTL) immune response of MS patients to self and to viral antigens. As self antigens, we used the nonamer peptide epitopes present on the myelin basic protein at amino acid position 110 to 118 (MBP_{p110}) and on the proteolipid protein at amino acid position 45 to 53 (PLP_{p45}). As viral antigens, we used peptides overlapping the entire amino acid sequence of

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VP1, the major capsid protein of the polyomavirus JC (JCV). MBP_{p110} and PLP_{p45} CTL epitopes are restricted by two different HLA alleles, the HLA-A*0201 and the HLA-A*0301 alleles, respectively (Honma et al., 1997; Tsuchida et al., 1994; Zhang et al., 2004). $CD8^+$T cells reactive against these epitopes exhibited MHC class I-restricted specific cytotoxicity toward autologous target cells pulsed with these auto-antigen myelin epitopes (Jurewicz et al., 1998).

As viral antigens, we chose to study the CTL response against JCV VP1. JCV is the etiologic agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the CNS, which occurs in the context of severe immunosuppression, such as in patients with AIDS, hemopathies, or in organ transplant recipients. We chose this virus for two reasons: first, it causes a latent infection in 85% of healthy adults, and therefore, the likelihood that MS patients would be infected was high; second, the frequency of JCV-specific CTL in non-PML patients varies from <1/100,000 to 1/2494 PBMCs (Du Pasquier et al., 2004). This low frequency is in the expected range of the magnitude of the CTL response against auto-antigen myelin epitopes (Tsuchida et al., 1994; Zhang et al., 2004).

We found that the JCV-specific CTL response in MS patients was more frequently detectable than the one against auto-antigen myelin epitopes MBP_{p110} and PLP_{p45}. This JCV-specific cellular immune response was also more vigorous in MS patients than in healthy control (HC) subjects.

2. Material and methods

2.1. Selection of the study subjects

We enrolled 18 MS patients in this study. At the time of enrolment, 12 patients had clinically definite MS (CD-MS), including 10 with relapsing–remitting (RR-MS), 1 with secondary progressive (SP-MS), and 1 with progressive-relapsing (PR-MS). In addition, six patients had possible MS at the time of the enrolment and indeed were subsequently diagnosed with RR-MS. To examine only MS patients with unbiased cellular immune response, those who had received corticosteroids, immunosuppressors or immunomodulatory medication in the past 3 months were excluded from the study.

The results of MS patients were compared to those of eight healthy control subjects (HC). Detection of JCV $VP1_{p36}$- and JCV $VP1_{p100}$-specific $CD8^+$T cells by tetramer staining and ^{51}Cr release assay had already been performed in four of them for a previous study (Du Pasquier et al., 2004). However, assessment of the cellular immune response against any potential epitope of VP1, using a ^{51}Cr release assay with pools of peptides encompassing the whole amino acid sequence of VP1 was performed specifically for the present study. The four remaining HC subjects were enrolled for this study and had never been reported before.

2.2. MHC class I typing

The MHC class I alleles expressed by all study subjects were determined using standard serologic tissue-typing procedures. In HLA-$A2^+$ or -$A3^+$ subjects, molecular analyses were performed to specify the subtypes.

2.2.1. Detection of HLA-A*0201-restricted MBP_{p110}- and HLA-A*0301-restricted PLP_{p45}-specific CTL by ^{51}Cr release assay

To determine the magnitude of the CTL response against auto-antigen myelin proteins, we synthesized two epitopes that had been previously described: the HLA-A*0201-restricted MBP_{p110} (SLSRFSWGA) (Tsuchida et al., 1994) and the HLA-A*0301-restricted PLP_{p45} (KLIETYFSK) (Honma et al., 1997). Using ^{51}Cr release assays, we tested for the presence of MBP_{p110}- and PLP_{p45}-specific CTL in 4 HLA-$A*0201^+$and 6 HLA-$A*0301^+$MS patients, respectively. One patient, who was HLA-$A*0201^+$and HLA-$A*0301^+$, was tested for both peptide epitopes.

2.2.2. Detection of JCV VP1-specific CTL in all subjects

The CTL response against VP1, the major capsid protein, was tested in all 18 MS patients and eight HC subjects by stimulating PBMC with pools of overlapping peptides encompassing the entire amino acids sequence of this protein. After 10–14 days of in vitro stimulation in the presence of recombinant human interleukin 2 (IL-2), these PBMC were tested in a ^{51}Cr release assay using autologous B lymphoblastoid cell lines (B-LCL), pulsed with the same pools of peptides, as target cells. With this technique, any possible nonamer CTL epitope of JCV VP1 protein could be detected (Du Pasquier et al., 2003).

2.2.3. Detection of JCV VP1-specific $CD8^+$ T cells in HLA-$A*0201^+$ subjects

HLA-$A*0201^+$MS patients, were tested for the presence of $CD8^+$T cells specific for two HLA-A*0201-restricted nonamer epitope peptides of the JCV VP1 protein, $VP1_{p36}$-SITEVECFL- and $VP1_{p100}$-ILMWEAVTL (Du Pasquier et al., 2003; Koralnik et al., 2002). PBMC of the study subjects were stimulated in vitro in presence of the two HLA-A*0201-restricted peptide epitopes and IL-2. After 10–14 days of such in vitro stimulation, JCV $VP1_{p36}$ or JCV $VP1_{p100}$ were detected using a tetramer staining assay as described in detail in previous publications (Du Pasquier et al., 2003; Koralnik et al., 2002).

2.3. Determination of JCV load in the plasma, PBMC and CSF

Plasma, PBMC and CSF from MS patients were harvested. Determination of JC viral load was performed by quantitative PCR (Q-PCR) using a pair of highly specific Q-PCR primers in JCV VP2 gene, which only amplify JCV sequence and do not cross-react with human genomic DNA,