



Immunovirology Report

JCV-specific cellular immune response correlates with a favorable clinical outcome in HIV-infected individuals with progressive multifocal leukoencephalopathy

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Most immunosuppressed individuals who develop progressive multifocal leukoencephalopathy (PML) have a rapid fatal outcome, whereas some become long-term survivors. We explored the impact of the cellular immune response against JC virus (JCV) on the clinical outcome of 7 HIV+ and 3 HIV- individuals with PML. Of the 4 HIV+/PML survivors, all had detectable cytotoxic T lymphocytes (CTL) specific for JCV T or VP 1 proteins compared to none of the 3 HIV+/PML progressors tested. Of the 3 HIV- /PML patients, 1 was recently diagnosed with PML and showed evidence of neurologic improvement without any treatment. This patient had CTL specific for the VP1 protein of JCV. The other 2 HIV- /PML survivors were stable 3–8 years after the diagnosis of PML. They did not have any detectable CTL against JCV. These findings suggest that JCV-specific immune response is associated with favorable outcome in HIV+ individuals with PML. The lack of detectable JCV-specific CTL in 2 HIV- /PML survivors might indicate a burnt-out disease without sufficient antigenic stimulation to maintain the cellular immune response. The detection of JCV-specific CTL in an HIV- patient recently diagnosed with PML, who was showing evidence of neurological improvement without any treatment, indicates that this finding may be used as a favorable prognostic marker of disease evolution in the clinical management of patients with PML. As the quest for an effective treatment of PML continues, JCV-specific cellular immune response deserves further attention because it appears to play a crucial role in the prevention of disease progression. *Journal of NeuroVirology* (2001) 7, 318–322.

Keywords: progressive multifocal leukoencephalopathy (PML); human immunodeficiency virus (HIV); acquired immune deficiency syndrome (AIDS); cytotoxic T lymphocytes

Our knowledge of the immune response to JCV is limited. Seroconversion occurs in childhood (Walker

and Padgett, 1983), and IgG antibodies specific for JCV can be detected in approximately 90% of the normal adult population by a hemagglutination inhibition assay or ELISA (Weber *et al*, 1997). Intrathecal synthesis of anti-JCV VP1 protein IgG antibodies has been detected in 76% of PML patients. However, there were no clinical or biological differences in these patients compared to those without detectable JCV-specific antibodies in the CSF (Weber

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et al, 1997). Two patients with PML had a fatal outcome despite a rise in CSF JCV-specific antibodies (Berner *et al*, 1999; Guillaume *et al*, 2000). In addition, individuals with PML have no detectable IgM antibodies in the serum or CSF. Finally, JCV-specific antibodies do not prevent virus excretion in the urine in immunocompetent individuals (Coleman *et al*, 1983).

JCV reactivation occurs in the context of immune suppression and humoral immunity is unable to control JCV spread. Therefore, cell-mediated immunity may play a role in the containment of JCV. However, studies of this immune response have been limited and date from before the beginning of the AIDS epidemic (Ellison, 1969; Knight *et al*, 1972; Marriott *et al*, 1975; Horn *et al*, 1978; Mathews *et al*, 1976; Willoughby *et al*, 1980). More recently, the major histocompatibility complex (MHC) class I and II molecules were found to be expressed at high levels within PML lesions. This finding suggests that an absence of antigen presentation due to decreased MHC expression could not explain the uncontrolled replication of JCV in the CNS (Achim and Wiley, 1992). We started to explore the cellular immune response against JCV and found that survivors of PML harbored specific cytotoxic T lymphocytes specifically directed against the T and VP1 proteins of JCV (Koralnik *et al*, 2001). In the present work, we compared the effects of JCV-specific cellular immune response with the patients' clinical outcomes.

Results

This study included 10 patients suffering from PML. The diagnosis was ascertained by clinical and neuroradiological criteria and confirmed by brain biopsy or by the presence of JCV-DNA in the cerebrospinal fluid as determined by PCR amplification. Of the 7 HIV+/PML patients, 4 were survivors whose disease had improved or remained stable 2–5 years after their initial diagnosis. Three were progressors, who had a fatal clinical outcome 7–25 weeks (mean: 15 ± 9 weeks) after the onset of their neurologic disease. Two of the 3 HIV-/PML patients were stable 3–8 years after their initial diagnosis. The third

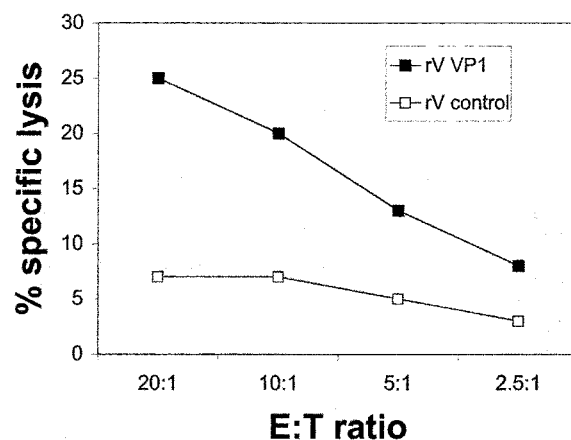


Figure 1 PBMC of an HIV-negative PML patient stimulated with autologous fixed B-LCL infected with a recombinant vaccinia virus expressing the JCV VP1 protein (rVVP₁). These PBMC lysed autologous target cells infected with the rVVP₁ (filled squares), but not with the wild-type vaccinia virus used as control (open squares). E: effectors. T: targets.

HIV-/PML patient was improving neurologically without any treatment 6 months after being diagnosed with PML.

As expected, the HIV+/PML survivors had a higher CD4+ cell count (140–771 cells/μl, mean 358) than the HIV+ individuals with fatal PML (9–104 cells/μl, mean 53), and a lower plasma HIV viral load (<50 copies/ml in 3 of 4 PML survivors, 3694 copies/ml in the fourth one) than the HIV+ with progressive disease (12,000–75,000 copies/ml).

The 10 patients were evaluated for the presence of JCV-specific CTL. Cytolytic activity specific for the JCV VP1 protein could be demonstrated in the peripheral blood mononuclear cells (PBMC) of the 4 HIV+/PML survivors and the HIV- patient recently diagnosed with PML (Figure 1). In addition, 3 out of 4 HIV+ / PML survivors had detectable cytolytic activity specific for JCV T protein. This cytolytic activity was mediated by CD8+ T cells, and was MHC class I-restricted, indicating that effectors were classical CTL (Koralnik *et al*, 2001). By contrast, the 2 HIV-/PML survivors showed no CTL activity against JCV proteins. None of the 3 HIV+/PML progressors had detectable CTL against JCV proteins (Table 1).

Table 1 Correlation of clinical outcome of PML patients with JCV-specific cellular immune response

Cellular immune response against JCV/ diagnosis	HIV+/PML survivors N = 4	HIV-/PML survivors N = 2	HIV-/PML early N = 1	HIV-/PML progressors N = 3
JCV T-specific CTL # pos/# tested	3/4	0/2	0/1	0/3
JCV VP1-specific CTL # pos/# tested	4/4	0/2	1/1	0/3

HIV+, HIV-positive; HIV-, HIV-negative; PML early, patient diagnosed with PML 6 months prior to the evaluation of her immune response, CTL, cytotoxic T lymphocytes.

At the time of the PML diagnosis, 5 of 7 HIV+ patients (3 progressors and 2 survivors) were already on HAART defined by at least 3 anti-retroviral medications including 1 or more protease inhibitors. Four were HAART-naïve patients (2 progressors and 2 survivors). Once the diagnosis of PML was established, all were treated with HAART. Four HIV+/PML patients (2 survivors and 2 progressors) received zidovudine in addition to HAART. Zidovudine was administered using the same regimen as described elsewhere (Meylan *et al*, 1999). Of the 2 HIV-/PML survivors, 1 had a history of non-Hodgkin's lymphoma and had received Ara-C for treatment of PML. The other had a history of a thymoma and was treated for PML with alpha interferon. They had been stable for 3 and 8 years, respectively, since the onset of their neurologic disease. The third HIV-/PML patient was a 80-year-old lady with a history of quiescent non-Hodgkin's lymphoma diagnosed 3 years prior to the onset of PML. She presented with severe cognitive dysfunction, global aphasia, and gait apraxia. Six months after her PML diagnosis, she was able to speak short sentences, follow simple commands, and interact with her caregivers, without having received any treatment. Unfortunately, she passed away 6 weeks later from sepsis secondary to a urinary tract infection and a decubitus ulcer.

Discussion

Our results suggest that the presence of JCV-specific CTL response is associated with long-term survival in HIV+/PML patients. Interestingly, we were also able to observe a strong cellular immune response directed against the JCV-VP1 protein in an HIV- patient who was recently diagnosed with PML, and who improved significantly without treatment. This observation suggests that CTL emergence may occur early in the course of the disease and could therefore lead to a better clinical outcome in PML patients.

By contrast with the HIV+/PML survivors, the 2 other HIV-/PML survivors did not show any JCV-specific CTL activity. The fact that they had been stable for 3 and 8 years after the onset of their neurologic disease indicates that they most likely had a burnt-out disease without sufficient antigenic stimulation to maintain the cellular immune response. Furthermore, it is possible that the functional lysis assay using vaccinia virus recombinant is not sensitive enough to detect very low level of circulating JCV-specific CTL in these patients. Alternatively, their cellular immune responses may be directed against the other JCV proteins: VP2, VP3, small t, or agnoproteins. None of the 3 HIV+/PML progressors tested had any specific CTL activity against JC virus. Therefore, the presence of JCV-specific CTL appears to be a useful prognostic marker of disease evolution.

All the HIV+/PML patients received HAART, defined as at least 3 medications, including a pro-

tease inhibitor. To date, HAART is the only treatment that has been found to improve the life expectancy of HIV+/PML patients, with an average survival of 46 weeks (Clifford *et al*, 1999; Gasnault *et al*, 1999; Miralles *et al*, 1998; Tassie *et al*, 1999). The effect of HAART on PML is thought to be through immune reconstitution. Indeed, in the HIV+/PML patients who respond to HAART, it is possible to observe a rise in CD4+ cell count as well as a drop in HIV viral load, paralleling the clinical improvement of their neurological disease. However, approximately 50% of the HIV+/PML patients do not show any improvement of their neurologic disease on HAART despite evidence of suppression of HIV replication and a rise of CD4+ counts (P Cinque, personal communication, the Biology of JC virus and PML workshop, Chicago, January 2001). Therefore, a rise of CD4+ cell count in itself does not appear to be a good prognostic marker of PML evolution. In this context, our data suggest that the presence of JCV-specific CTL represents a better immune surrogate marker of PML prognosis in HIV+/PML patients.

Four patients (2 survivors and 2 progressors) received zidovudine in addition to HAART. Although, the number of patients treated with zidovudine is too small to draw any definitive conclusion about the efficacy of this medication, it is interesting to notice that the 2 survivors received zidovudine long after the beginning of their symptoms (mean 27 weeks), while they were already improving under HAART or concomitantly with a change of HAART composition. By contrast, the 2 progressors received zidovudine sooner (mean 7.5 weeks). Thus, if this drug was efficient, one would rather have expected a favorable outcome in these 2 patients than in the 2 survivors, due to a shorter delay between diagnosis and onset of treatment. Yet, the clinical outcome of the 2 progressors was rapidly fatal, leading to death in 1 to 6 months. Neither immune reconstitution, as measured by CD4+ cell counts, nor complete suppression of HIV replication, could be achieved in these patients with the combination of HAART and zidovudine.

As the quest for an effective treatment for PML continues, JCV-specific cellular immune response deserves further attention since it appears to play a crucial role in the prevention of disease progression. Prospective studies are now ongoing in our institution to determine if the detection of JCV-specific CTL early on in the course of the disease is indeed a favorable prognostic marker of disease evolution.

Functional lysis assays using the vaccinia virus recombinants described in this study allow the expression of the entire viral protein of interest in autologous B lymphoblastoid cell lines (BLCL) used as target cells. The viral protein produced inside of the target cell is then degraded and processed via the endogenous pathway for presentation to the T-cell receptors of the CTL. CTL recognizes virus-infected cells through the interaction of their T-cell

receptor and a viral peptide epitope presented by the MHC class I molecule of the infected cell. The viral CTL epitope is usually a 9-aa fragment of a viral protein with specific chemical affinity for the peptide-binding site of an MHC class I molecule. The MHC class I molecule/viral nonamer epitope complex forms in the endoplasmic reticulum of the infected cell, and then migrates to the cell surface (Klein and Sato, 2000). Following recognition by peptide-specific CTL, the infected cell is then eliminated through a variety of mechanisms including the secretion of perforin and granzyme by the CTL.

The advantage of the vaccinia virus recombinant technique used in this study is that it reproduces a real-life situation as it allows the association of every possible CTL epitope of the viral protein of interest to associate with the various MHC class I molecules expressed by the target cells of any given patient. The drawback of this technique lies in the necessity to derive and expand a BLCL from each study subject. In end-stage AIDS patients, this can take up to 2 months. This technique is also work-intensive and requires the use of radioactive isotopes. Therefore, it is not suitable for the study of large groups of patients, most of whom may have a survival time of only several months.

Further characterization of the CTL epitopes of JCV is now necessary for devising adequate screening assays as well as strategies to enhance the cellular immune response against this virus in patients with PML, or in those who are at risk of developing this disease. Studies now in progress in our laboratory involve computer predictive analysis of JCV CTL epitopes based on their calculated binding affinity to specific MHC class I molecules, as well as epitope-mapping studies using overlapping peptides spanning entire JCV proteins.

The characterization of JCV CTL epitopes will allow the construction of fluorochrome-tagged MHC class I/JCV peptide tetramer complexes, which will then be used to stain JCV-specific CTL directly from peripheral blood or in cultured cells. This technique does not require the use of radioactive compounds or BLCL, and facilitates detection and quantification of JCV peptide-specific CTL directly by flow cytometry (Altman *et al*, 1996). JCV-specific CTL populations could also be enriched using tetrameric MHC class-I/JCV peptide complexes. An expanded autologous population of such cells would be extremely valuable as a therapy for individuals with

PML. T-cell transfer therapy has already been used successfully for the treatment of EBV and CMV infections in bone marrow transplant recipients (Papadopoulos *et al*, 1994; Walter *et al*, 1995). Another way to boost the immune response against JCV would be to use a plasmid DNA vaccine encoding the CTL epitopes. Such a vaccine could be used prophylactically in patients with AIDS who are at risk of developing PML, or as therapy for PML. A similar approach is currently being tested for the treatment of melanoma (Weber, 2000). These vaccinations might well augment JCV-specific cell-mediated immunity and block the progression of JCV-induced disease.

Methods

Cytotoxic T lymphocyte assay

In developing an assay for JCV-specific CTL, we generated recombinant vaccinia-JCV constructs to express JCV T and VP1 gene products in autologous B-lymphoblastoid cell lines (BLCL), which were made to serve as both stimulator and target cells. Stimulators were prepared by infecting autologous BLCL with vaccinia viruses expressing either the T or VP1 JCV genes, followed by fixation in 1.5% paraformaldehyde. The same recombinant vaccinia viruses were used to express the JCV T or VP1 genes in autologous BLCL. Effectors were generated by mixing the patient's PBMC with autologous stimulator cells at a ratio of 1:1 in presence of interleukin-2 for 11 to 14 days. The stimulated cells were then added at various effector-to-target ratios to ⁵¹Cr-labeled autologous target cells. After 4 h of incubation, supernatants were harvested and their radioactivity was measured (Koralnik *et al*, 2001).

PCR detection for JCV DNA

Extraction of DNA from CSF and quantitative PCR for JCV were performed as described previously (Koralnik *et al*, 1999).

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