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Rapid Communication

Efficient *in vitro* expansion of JC virus-specific CD8$^+$ T-cell responses by JCV peptide-stimulated dendritic cells from patients with progressive multifocal leukoencephalopathy

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ABSTRACT

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the brain caused by JC virus (JCV) for which there is no cure. PML patients who have JCV-specific CD8$^+$ cytotoxic T lymphocytes (CTL) in their blood have a better clinical outcome. We compared JCV-specific CTL responses *in vitro* elicited either by JCV peptide-loaded dendritic cells (DC) or by direct peptide stimulation of lymphocytes from 20 HLA-A*0201$^+$ healthy controls, HIV$^+$ and PML patients. JCV peptide-loaded DC elicited a stronger CTL expansion in 13/15 responders. DC can induce a potent JCV-specific CTL response *in vitro*, and may constitute a promising approach for PML immunotherapy.

Introduction

JC virus is the agent of progressive multifocal leukoencephalopathy (PML) (Koralnik, 2006) which occurs in up to 5% of people with AIDS (Antinori et al., 2003), as well as in patients with lymphoproliferative disorders, organ transplant recipients and individuals treated with natalizumab (Berger and Koralnik, 2005). After asymptomatic primary infection, JCV remains quiescent in the kidney, and can be found in urine of 30% of healthy and immunosuppressed individuals alike (Koralnik et al., 1999) and in lymphoid organs (Monaco et al., 1998).

In immunocompromised individuals, JCV may reactivate from sites of latency and causes a lytic infection of oligodendrocytes leading to multiple areas of demyelination in the central nervous system, and ultimately death within a few months. There is no effective treatment for this disease and those patients who survive are often left with devastating neurological sequellae (De Luca et al., 1998).

Therefore, a therapeutic vaccine may contain JCV replication, especially in the early stage of the disease, and may reduce the morbidity and mortality in patients with PML.

The antibody response to JCV is unable to prevent development of PML or prevent disease progression (Weber et al., 1997). Conversely, the cellular immune response mediated by JCV-specific CD8$^+$ cytotoxic T-lymphocytes (CTL) plays a crucial role in the containment of PML (Du Pasquier et al., 2003; Du Pasquier et al., 2004; Koralnik et al., 2002). Hence, a treatment that improves the CTL response against JCV may be of benefit in patients with PML. Such therapies may consist of adoptive transfer of T cells after *ex vivo* stimulation with JCV antigens or dendritic cells (DC)-based immunotherapy (June, 2007; O'Neill and Bhardwaj, 2005). DC are the professional antigen-presenting cells in the body. A small number of DC and low level of antigens are able to induce a potent CD8$^+$ T-cell response *in vitro* and *in vivo* (Adams et al., 2005). In addition, the safety of DC immunization has been established in numerous clinical trials (Dhodapkar and Bhardwaj, 2000). Furthermore, promising trials of DC vaccination have already been reported in patients with HIV infection (Lu et al., 2004).

The frequency of JCV-specific CTL in fresh blood is very low, and their function cannot usually be measured *ex vivo* by conventional assays without prior stimulation with JCV antigens (Lima et al., 2007). In our quest for a treatment for PML, we first developed a DC-based stimulation of JCV-specific T cells. Then, to determine the respective potential of autologous transfer of T cells and DC-based immunotherapy for PML, we compared the CTL response elicited *in vitro* from peripheral blood lymphocytes against JCV CTL epitopes by direct

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peptide stimulation, as performed in adoptive transfer of T cells, and by JCV-peptide loaded mature monocyte-derived DC.

Results

Peripheral blood samples were obtained from 20 JCV seropositive HLA-A*0201$^+$ subjects, including 6 with PML (5 HIV$^+$ and 1 HIV$^-$) aged 40 to 69 years (47.8±12.1 years [mean±SD]), 4 HIV$^+$ individuals aged 44 to 50 years (45.5±3 years) and 10 healthy controls (HC) aged 24 to 44 (34±8 years). HIV$^+$/PML and HIV$^+$ patients had a mean CD4$^+$ T cell count of 521/μL±289 and 496/μL±320 respectively, and a plasma HIV RNA level of 1.25 $\log_{10}$ copies/ml±0.5 and 1.98 $\log_{10}$ copies/ml±1.9, respectively. All HIV$^+$ and HIV$^+$/PML patients were on highly active antiretroviral therapy (HAART). All but one PML patients were still alive an average of 9.2 years (3.4 to 15.2 years) after disease onset.

JCV-specific CTL responses against $VP1_{p36}$ and/or $VP1_{p100}$ epitopes, were detected in 14/20 study subjects, including 5/6 PML patients, 3/4 HIV$^+$ individuals and 6/10 HC after direct peptide stimulation of lymphocytes. These responses were enhanced using peptide-loaded DC stimulation in 14/16 comparisons performed in these 14 individuals (Table 1). The average amplification factor of JCV-specific CD8$^+$ T-cell response using peptide-loaded DC compared to peptide stimulation alone was 6.8× for PML patients, 3.9× for HIV$^+$ patients, and 31.0× for HC. Furthermore, we measured a JCV epitope-specific CTL response after DC stimulation in 1 HIV$^+$ individual (#1, $VP1_{p100}$) and 2 HC (#3, $VP1_{p100}$ and #9, $VP1_{p36}$) who had no detectable CTL after stimulation of lymphocytes with peptide alone. The median percentage of JCV-specific CD8$^+$ T cells in peptide alone and peptide-loaded-DC stimulation of lymphocytes was 0.7% (0.1–40.1%) vs 7.3% (0.2–48.8%) in the whole study population including both JCV $VP1_{p36}$ and $VP1_{p100}$ epitopes. There was a significant increase in the JCV-specific CTL response for both epitopes in peptide-loaded DC versus peptide alone stimulation of lymphocytes in all 15 responders ($p=0.004$, Wilcoxon matched-paired test).

In 1 PML patient and 4 HC, we did not observe any JCV-specific CTL response using either method. Strong CTL responses to positive control Flu matrix protein (MP) peptide were elicited using peptide-loaded DC stimulation in all tested cases, while no responses were elicited against negative control NY ESO cancer peptide, as expected (data not shown). A representative example of CTL response elicited by direct peptide or peptide-loaded DC stimulation of lymphocytes is shown in Fig. 1.

Interestingly, among the PML group, we detected the strongest JCV-specific CTL response using DC, in PML patient #4 diagnosed only 2 months before testing. In contrast, PML #5 who developed PML 15.2 years before testing had no detectable JCV-specific CTL response with either peptide or peptide-loaded DC stimulation. Only two study subjects, PML patient #6 and HC #7 had a stronger CTL response against JCV epitopes using peptide alone compared to peptide-pulsed DC stimulation. A representative example of the increase in the JCV $VP1_{p36}$ or JCV $VP1_{p100}$-specific CTL response obtained using peptide-loaded DC compared to peptide alone stimulation of lymphocytes is shown in Fig. 2A, for one subject of each study group. To verify the function of the tetramer-staining cells, a ^{51}Cr release assay was performed after both types of stimulation in a sample from HC#1 (Fig. 2B). Only the peptide-loaded DC stimulation was potent enough to generate a sufficient number of functionally active effector CD8$^+$ T cells that could be detected by the ^{51}Cr assay. While peptide-specific CTL are usually undetectable in fresh blood samples in most individuals (Du Pasquier et al., 2003) our findings suggest that stimulation of peripheral blood lymphocytes *in vitro* with peptide-loaded autologous DC elicit in most cases a stronger CTL response than stimulation with peptide alone in individuals of the 3 study groups. The lack of any detectable JCV CTL expansion in lymphocytes of 4 HC

Table 1
Detection of JCV $VP1_{p36}$- and JCV $VP1_{p100}$-specific CD8$^+$ T cells in lymphocyte cultures stimulated with peptide-loaded autologous DC or peptide alone

Subjects	% JCV VP1p36-specific CD8$^+$ T cells		Amplification factor	% JCV VP1p100-specific CD8$^+$ T cells		Amplification factor
	Peptide alone	Peptide-loaded DC		Peptide alone	Peptide-loaded DC	
PML patient #						
1	0.1	0.4	4.0×	NA[a]	NA	
2	0.4	1.2	3.0×	2.2	16.5	7.5×
3	–[b]		∅	0.6	1.0	1.7×
4	1.0	24.5	24.5×	NA	NA	
5	–	–	∅	–	–	∅
6	40.1	3.6	0.1×	NA	NA	
HIV$^+$ patient #						
1	NA	NA		–	0.2	[c]
2	NA	NA		0.4	3.2	8.0×
3	NA	NA		3.6	8.0	2.2×
4	NA	NA		4.2	7.3	1.7×
Healthy control #						
1	0.7	48.8	69.7×	NA	NA	
2	0.2	15.0	75.0×	4.9	7.4	1.5×
3	0.8	22.1	27.6×		0.3	[c]
4	0.5	12.3	24.6×	NA	NA	
5	–	–	∅	NA	NA	
6	–	–	∅	NA	NA	
7	–	–	∅	8.6	6.6	0.7×
8	–	–	∅	NA	NA	
9	–	2.6	–[c]	2.6	47.1	18.1×
10	–	–	∅	NA	NA	

Notes. ∅, no amplification.
The amplification factor, defined as the ratio between frequency of JCV specific CTL frequency after stimulation with peptide-loaded DC versus peptide alone, and varies from 0.1 to 75× in patients with detectable JCV response.
[a] NA, not available.
[b] –, undetectable.
[c] JCV $VP1_{p36}$ and $VP1_{p100}$-specific CD8$^+$ T cells detectable after peptide-loaded DC stimulation only.