

JCV-specific T-cells producing IFN-gamma are differently associated with PML occurrence in HIV patients and liver transplant recipients

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SUMMARY

Aim of this work was to investigate a possible correlation between the frequency of JCV-specific T-cells and PML occurrence in HIV-infected subjects and in liver transplant recipients.

A significant decrease of JCV-specific T-cells was observed in HIV-PML subjects, highlighting a close relation between JCV-specific T-cell immune impairment and PML occurrence in HIV-subjects. Interestingly, liver-transplant recipients (LTR) showed a low frequency of JCV-specific T-cells, similar to HIV-PML subjects. Nevertheless, none of the enrolled LTR developed PML, suggesting the existence of different immunological mechanisms involved in the maintenance of a protective immune response in LTR.

KEY WORDS: JCV-specific T cells, PML, HIV subjects, Liver transplant recipients.

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Progressive multifocal leukoencephalopathy (PML) is a rare often fatal demyelinating disease of the central nervous system caused by the reactivation of the human polyomavirus JC (JCV) (Ferenczy *et al.*, 2012). Although asymptomatic JCV primary infection occurs in the large majority of the general population, reactivation resulting in PML is observed mainly in immunosuppressed individuals, including HIV-infected persons, cancer patients, organ transplant recipients, and patients undergoing lymphocyte-targeted drug treatment for autoimmune diseases (Ferenczy *et al.*, 2012).

In HIV infection, the PML-attributable 1-year mortality rate is greater than 50% in AIDS patients (Antinori *et al.*, 2003), with 80% of survivors showing persistent severe neurological impairment. Nevertheless, despite the lack of specific treatment, the immune reconstitution induced by combined antiretroviral therapy (cART) has significantly improved survival in AIDS patients (Antinori *et al.*, 2003). In the HIV population, JCV-specific T-cells have been reported to play a major role in containing JCV reactivation, and were associated with an improved PML clinical outcome (Du Pasquier *et al.*, 2001). Specifically, the early detection of JCV-specific CTL in HIV+ patients had an 87% predictive value for subsequent control of PML (Du Pasquier *et al.*, 2004). In contrast, PML non-survivors had selectively impaired JCV-specific T-cell responses (Khanna *et al.*, 2009).

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Organ transplant recipients represent a risk group for developing PML due to the immunosuppressive regimens. In this clinical setting, the survival rate remains extremely poor and the only available treatment option is reducing and/or terminating the immunosuppressive regimen (Mateen *et al.*, 2011; Shitrit *et al.*, 2005; Verhelst *et al.*, 2011).

The identification of a prognostic bio-marker able to identify patients with an increased risk for developing PML would represent a useful tool in the clinical management of immunosuppressed patients. Unfortunately, effective prognostic markers are still lacking. Active JCV replication in urine or plasma does not correlate with an increased risk of developing PML (Koralnik *et al.*, 1999). Notably, JCV-specific T-cells have been shown to play a major role in containing JCV reactivation, and have been proposed as a prognostic marker for HIV patients at risk of PML (Khanna *et al.*, 2009). No data are available on the possible predictive value of this marker in liver transplant recipients.

Aim of this work was to investigate a possible correlation between the frequency of JCV-specific T-cells and PML occurrence in two different immuno-compromised groups: HIV-infected subjects and liver transplant recipients (LTR).

Specifically, we enrolled:

- 1) PML-free HIV-infected patients (HIV-PML-free, n=16);
- 2) HIV-infected patients with a PML diagnosis (HIV-PML, n=4);
- 3) LTR recipients (n=11). Healthy donors (HD, n=10) were enrolled as controls.

Clinical features of enrolled patients are reported in Table 1A. All HIV patients were under cART treatment, and peripheral blood of HIV-PML subjects was obtained within 3 days from diagnosis. All LTR patients are under immunosuppressive regimens, and underwent liver transplant for HCV-related (n=7), HBV-related (n=2), and alcoholic-related (n=2) pathologies. Laboratory tests were performed using anonymised residual samples obtained for diagnostic purpose. The presence of JCV-DNA in the cerebrospinal fluid together with evidence of demyelinating white matter lesions at magnetic resonance imaging in patients with compatible clinical features were considered diagnostic for PML (Berger *et al.*, 2013). JCV-DNA was quantified by real time PCR (Koralnik *et al.*, 1999).

As described in Table 1A, although not reaching statistical significance, the CD4 T cell number was lower in HIV-PML subjects compared to HIV-PMLfree (HIV-PML: 245 ± 179 vs. HIV-PMLfree: 545 ± 121 , $p > 0.05$). Moreover, all HIV-

TABLE 1 - Patients

1A - Enrolled subjects						
Patients	N	Gender (M/F)	Mean Age (years)	Mean CD4/mm ³	Plasma HIV Viral load (VL) (cp/ml)	Treatment
HD	10	5/5	54.7	n.t.	n.t.	None
HIV-PMLfree	16	13/3	49.4	545 ± 121	<40 (11 patients) 40<VL<1000 (3 patients) VL >10000 (2 patients)	cART
HIV-PML	4	4/0	51.7	245 ± 179	<40 (2 patients) 40<VL<60 (2 patients)	cART
LTR	11	9/2	55.3	n.t.	n.t.	Tacrolimus (n=10) Everolimus (n=1)
1B - Immunological and virological features of HIV-PML subjects						
HIV-PML patients	CD4 (CD4/mm ³)	CSF JCV-DNA (cp/ml)	CSF HIV-RNA (cp/ml)	JCV-specific T-cells (SFCs/10 ⁶ PBMC)	Outcome at 2 years	
#1	115	2144	Undetected	68	survivor	
#2	334	392	54	40	nonsurvivor	
#3	77	150	Undetected	0	nonsurvivor	
#4	454	249	Undetected	83	survivor	

n.t.: not tested; CSF: cerebrospinal fluid.

PML patients showed undetected/low HIV plasma viral load, suggesting that PML occurrence was not associated with high HIV replication. Finally, HIV-RNA as well as JCV-DNA was tested in CSF (Table 1B). HIV-RNA was detected at low copies only in 1/4 HIV-PML and JCV-DNA ranged between 150-2144 cp/ml. HIV-PML survival outcome was defined at the end of 2 years follow-up. Neither CD4 T cell number nor HIV-RNA (either in plasma and in CSF), nor JCV-DNA viral load in CSF were associated with the outcome of PML (survivors/non-survivors). JCV-specific T-cell immunity was analysed by ELISpot assay. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and stimulated for 20 hours with peptides from VP1 and LT1 JCV proteins in the presence of 1 μ g/ml α CD28 and α CD49d (Becton Dickinson, Mountain View, CA, USA). PHA stimulation was used as a positive control to assess the functionality of immune cells, and all enrolled patients showed a good response (data not shown). Spontaneous (background) cytokine production was assessed by incubating PBMC with α CD28 and α CD49d. Results were expressed as spot-forming cells (SFC)/10⁶ PBMCs in stimulated cultures, after having subtracted background. Statistical analysis was performed using non-parametric Mann-Whit-

ney test. Differences were considered significant when the p-value was less than 0.05. Statistical evaluations were performed by GraphPad Prism software (GraphPad).

As shown in Figure 1, a similar frequency of JCV-specific T-cells was detected in the peripheral blood of HD and HIV-PMLfree subjects [HD: 100 SFC/10⁶ PBMC (IQR: 71-158) vs HIV-PMLfree: 136 SFC/10⁶ PBMC (IQR: 66-209)]. By contrast, HIV-PML subjects showed a significant decrease in the frequency of JCV-specific T-cells when compared to both HD [HIV-PML: 54 SFC/10⁶ PBMC (IQR: 20-75.5) vs HD: 100 SFC/10⁶ PBMC (IQR: 71-158), $p < 0.05$] and to HIV-PMLfree patients [HIV-PML: 54 SFC/10⁶ PBMC (IQR: 20-75.5) vs HIV-PMLfree: 136 SFC/10⁶ PBMC (IQR: 66-209), $p < 0.05$], suggesting a close relation between JCV-specific T-cell immune impairment and PML occurrence in HIV-infected patients. Moreover, a positive JCV-specific T-cell response was found in 2/2 (100%) of HIV-PML survivors and in 1/2 (50%) of HIV-PML non-survivors (Table 1B). These data confirm published results, highlighting the major role of JCV-specific T cells in disease containment and survival during PML in an HIV clinical setting (Du Pasquier *et al.*, 2001; Du Pasquier *et al.*, 2004; Khanna *et al.*, 2009). Indeed, this factor has been proposed as a prog-

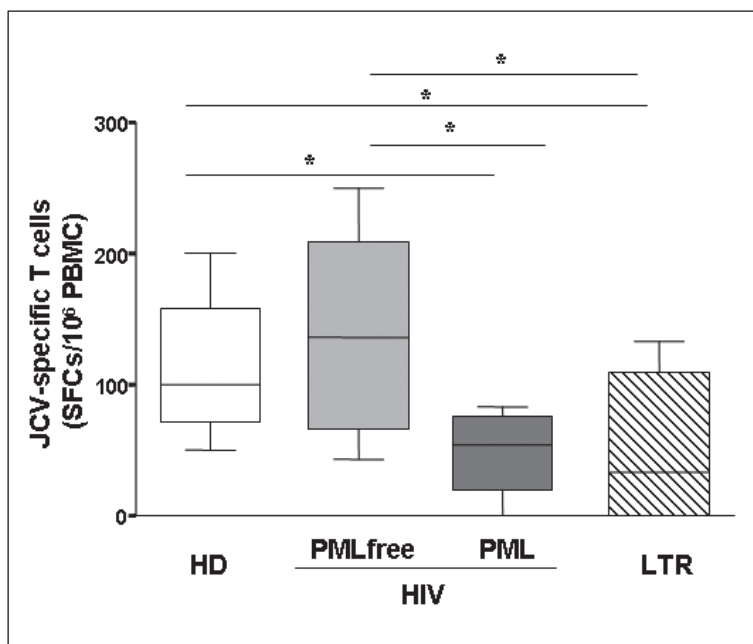


FIGURE 1 - JCV-specific T cell frequency. JCV-specific T-cells are analysed in HD (n=8), HIV-PMLfree (n=12), HIV-PML (n=4) and LTR (n=11) by ELISpot assay. Results are expressed as SCFs/10⁶ PBMC.

nostic marker for HIV patients at risk of PML (Khanna *et al.*, 2009).

In order to verify whether JCV-specific T-cell immunity may be used as a marker associated with PML occurrence also in another highly immunosuppressed clinical settings, LTR were enrolled. LTR showed a low frequency of JCV-specific T-cells, similar to HIV-PML subjects [LTR: 33 SFC/10⁶PBMC (IQR: 0-109.5) vs. HIV-PML: 54 SFC/10⁶PBMC (IQR: 20-75.5)]. Interestingly, none of the enrolled LTR developed PML, suggesting that unlike HIV-infected subjects, a low frequency of JCV-specific T-cells was not associated with the onset of PML in LTR. Despite the small sample size and the lack of an LTR-PML group, our results may suggest different immunological mechanisms able to drive a protective immune response in HIV and LTR.

Several immunological mechanisms can be invoked to explain the different behaviour of HIV and LTR patients, as polyfunctional properties and/or exhaustion/regulation/trafficking marker expression. In this study, JCV-specific T-cells were identified as cells able to produce IFN- γ after JCV specific stimulation. Nevertheless, it is well-known that virus-specific T-cells exert their antiviral functions by producing a large panel of cytokines/chemokines and also by mediating a direct cytotoxic activity. Indeed, it is also well-accepted that a polyfunctional profile of T-cells rather than a simple function is associated with protection in viral infections (Seder *et al.*, 2008). Moreover, the expression of exhaustion/regulation markers may be involved in regulating the cytokine profile and thus modulating the protective efficacy (Tan *et al.*, 2012). Thus, we may speculate that in LTR, polyfunctional/IFN- γ negative JCV-specific T-cells were able to maintain a sufficient level of protective immunity to avoid JCV reactivation. Finally, L-selectin expression on CD4 T cell surface has been proposed as a marker associated with PML in HIV patients (Schneider-Hohendorf *et al.*, 2014).

New studies are necessary to verify the hypothesis that other T cell functions (e.g., perforin expression, TNF- α , IL-2 production, etc) as well as the expression of biomarkers may be used to evaluate LTR at risk of PML. Although representing an extremely rare population (Verhelst *et al.*, 2011), work is in progress on the enrol-

ment of LTR-PML subjects. This may represent a critical step to dissect immunological protective mechanisms in the clinical transplant setting. Overall, this study showed that the quantification of JCV-specific T-cells only by IFN- γ production was not a useful marker to identify LTR patients with PML.

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Conflict of interest

The authors disclose no financial or personal relationships with other people or organizations that could inappropriately influence the work.

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