Journal of NeuroVirology, 13: 73–77, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280601094563

informa
healthcare

Short Communication

JC virus viremia in interferon-β–treated and untreated Italian multiple sclerosis patients and healthy controls

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Following the development of progressive multifocal leukoencephalopathy (PML) in two multiple sclerosis (MS) patients treated with natalizumab and interferon-β (IFNβ), a possible correlation between JC virus (JCV), the etiological agent of PML, and MS has received heightened interest. In particular, attention has focused on assessing whether IFNβ treatment could affect the replication of JCV and thus its frequency in the peripheral blood of MS patients and whether the presence of JCV DNA in peripheral blood could be a predictive marker of the risk of developing PML. In order to answer to these questions, peripheral blood samples were collected from 59 INFβ-treated, 39 untreated relapsing-remitting MS patients, and 98 healthy controls (HCs) and JCV DNA levels were determined and quantified by means of a real-time polymerase chain reaction (Q-PCR) assay. Overall, no differences were found in the presence or viral load of JCV DNA of MS patients and the HCs, but JCV DNA was significantly less frequent in the peripheral blood of IFNβ-treated patients (13.6%) compared to the untreated MS patients (46.1%) and the healthy controls (28.6%). These results suggest that the presence of JCV in the blood of MS patients cannot be considered as a marker or a risk factor for PML development. In addition, they indicate that treatment with INFβ can lead to the reduction of presence of the JCV genome in the peripheral blood of MS patients and, thus, that this drug probably does not increase the risk of PML in MS patients treated with IFNβ. *Journal of NeuroVirology* (2007) **13,** 73–77.

Keywords: $\alpha 4\beta 1$ integrin; interferon-β; JC virus; multiple sclerosis; PML

Introduction

JC virus (JCV) is a human polyomavirus that during childhood infects about 80% of the population worldwide, without causing any disease (Walker and Padgett, 1983). After the primary infection, JCV can establish latency in the kidneys, bone marrow, tonsils, and spleen (Atwood *et al*, 1992; Monaco *et al*, 1996, 1998; Caldarelli-Stefano *et al*, 1999) and, after reactivation in immunocompromised individuals, the virus can cause progressive multifocal leukoencephalopathy (PML). PML is a fatal demyelinating disease of the white matter of the brain, due to the lytic destruction of oligodendrocytes infected by JCV (Zu Rhein, 1967; Padgett *et al*, 1971).

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), with various clinical patterns, including the relapsing-remitting and chronic progressive forms. The etiology of this autoimmune disease is still unknown, but genetic and environmental factors are thought to play significant roles in its pathogenesis, with the suspected

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This work was supported by NIH grant MH072528 and by a grant from the Italian Ministry of Health (ISS 2004) awarded to PF.

Received 13 July 2006; revised 20 October 2006; accepted 27 October 2006.

involvement of an as yet unidentified infectious agent. In particular, several viruses and bacteria have been suggested over time as possible triggering agents of MS (Granieri *et al*, 2001).

The possible involvement of JCV in MS pathogenesis was first postulated by Stoner (1991). Some studies (Bukle *et al*, 1992) exclude this possibility, whereas others (Ferrante *et al*, 1998; Olmos *et al*, 2005) report the presence of JCV DNA in the cerebrospinal fluid (CSF) of MS patients, but not of controls. Very recently, a correlation between MS and JCV unexpectedly emerged when two MS patients enrolled in a clinical trial with natalizumab (Tysabri; Biogen Idec/Elan), a humanized monoclonal antibody to $\alpha 4\beta 1$ integrin, and interferon-β (IFNβ), developed PML (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould *et al*, 2005).

This event led to immediate suspension of the treatment, but, due to the apparent efficacy of this drug, it has been readmitted for treatment, with a special restricted distribution program, in August 2006. This episode has led to an interesting scientific debate and the urgency of the many questions and issues involved has clearly emerged. Among such issues lies the importance of assessing the *in vivo* effect of IFNβ on JCV reactivation given that the two patients who developed PML during the clinical trial were also undergoing treatment with IFNβ. Moreover, a reliable, easy-to-use marker of JCV reactivation and thus of increased risk of developing PML would also constitute an essential tool of critical importance.

To gain further insight on the role of JCV and to contribute to this recent debate, a study was designed to evaluate the presence and the viral load of JCV DNA in the peripheral blood of INFβ-treated and untreated relapsing-remitting multiple sclerosis (MS) patients and healthy controls (HCs).

Results

The data obtained in this study indicated no difference in the prevalence of JCV DNA in the blood samples collected from relapsing-remitting MS patients and HCs (Table 1).

In Table 1, it is also possible to note that the frequency of JCV DNA in the blood samples was significantly lower in the IFNβ-treated MS patients than in the untreated MS cases ($P < .001$; odds ratio [OR] = 0.18; confidence interval [CI; 95%] 0.06–0.53).

Moreover, the presence of JCV DNA in the group of IFNβ-treated MS cases was also significantly lower than that one observed in the group of healthy control subjects ($P < .05$; OR = 0.39; CI (95%) 0.15–1.00).

The JCV viral load was also measured in all samples using real-time polymerase chain reaction (Q-PCR) in order to investigate the possibility that the levels of viral replication could differ between MS cases and controls and between IFNβ-treated and untreated MS patients. As reported in Figure 1, no significant differences were observed in these comparisons: the viral load was very similar in both groups of MS cases and in the healthy controls.

Discussion

The two cases of PML that occurred in a large group of MS patients treated with natalizumab and IFNβ determined the suspension of the use of this new immunomodulatory drug. After careful evaluation (Yousry *et al*, 2006), this drug is likely to be used in the treatment of MS again; however, given that the risk of PML has been estimated as 1 case per 1000 treated patients and that similar new drugs are likely to be available for MS treatment in the near future, several critical points need to be clarified.

Among others, two points are of particular relevance: (a) the need for a reliable, easy-to-use predictive marker of JCV reactivation and eventually of PML development and (b) the need to investigate whether combined treatment with IFNβ and natalizumab can increase the risk of developing PML.

JCV is a very common agent and is latent in the kidneys of a large part of the adult population worldwide. The virus is thought to establish latency also in B cells (Atwood *et al*, 1992; Monaco *et al*, 1996; Rieckmann *et al*, 1994). Therefore, our attempt focused on understanding whether JCV viremia may be associated with MS and possibly represent an indicator of susceptibility to PML development. The results presented here show that JCV DNA was detected with the same prevalence in the blood of the MS patients and the HCs. These findings confirm that

Table 1 Prevalence and mean viral load of JCV in blood of IFNβ-treated and untreated relapsing-remitting MS patients and of healthy controls

	Multiple sclerosis patients			
	IFNβ-treated $n = 59$	*Untreated* $n = 39$	*Total* $n = 98$	*Healthy controls* $n = 98$
JCV DNA + (%)	8 (13.6%)*,#	18 (46.1%)*	26 (26.5%)	28 (28.6%)#
JCV viral load (log copies/μg)	3.2	3.1	3.1	2.7

*IFNβ-treated versus untreated MS patients: $P = .00083$; odds ratio (OR) = 0.18; 95% CI: 0.06–0.53.
#IFNβ-treated versus healthy controls: $P = .044$; OR = 0.39; 95% CI: 0.15–1.00.

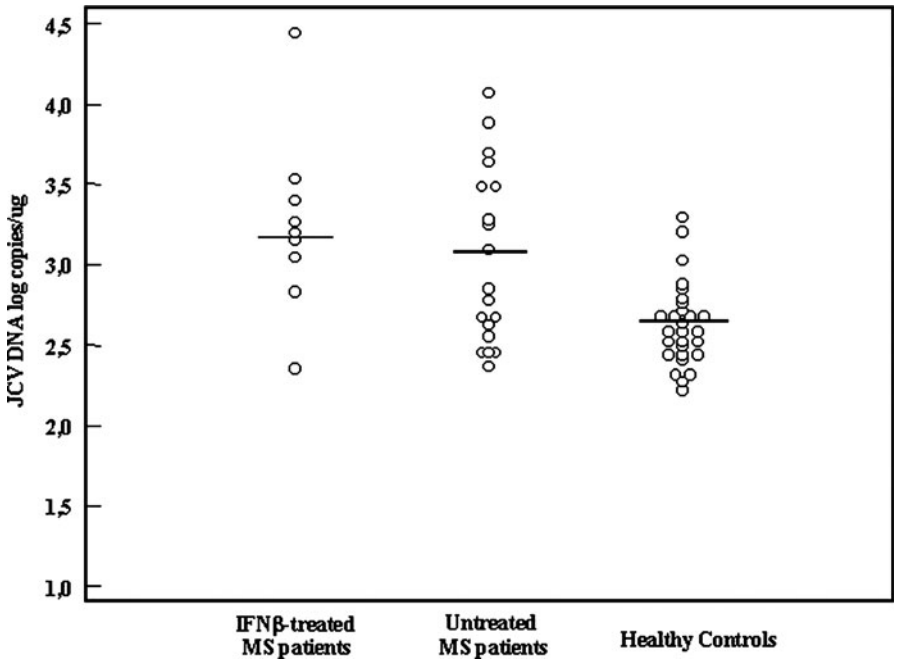

Figure 1 Plots of JCV DNA viral load (log copies/μg of DNA extracted from blood) as determined by Q-PCR for each individual of the three studied groups.

the presence of JCV in peripheral blood mononuclear cells (PBMCs) from immunocompetent subjects, including MS patients, is a relatively common event (Tornatore *et al*, 1992; Dorries *et al*, 1994; Ferrante *et al*, 1997; Pietropaolo *et al*, 2005).

Likewise, very similar viral load was found in peripheral blood of MS patients (both IFNβ-treated and untreated) and HCs, suggesting that this value does not provide any indication neither on the possibility of developing the pathology or on the effect of the treatment on the presence of JCV DNA in PBMCs.

Thus, on the basis of these results, it can be suggested that the presence of JCV DNA in MS patients cannot be considered as a valid predictive marker of the risk of developing PML.

IFNβ has seen widespread use in treating relapsing-remitting MS, because it induces a consistent reduction of the clinical relapse rate, without significant side effects, whether used alone or in combination with other drugs. It has been noted that INFβ also has immunosuppressive effects, even if its antiviral activity is well known.

Although all around the world many MS patients have been treated with IFNβ for years, PML cases had never been reported previously. However, because the two PML cases observed during the above-mentioned clinical trial were also treated with IFNβ (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould *et al*, 2005), it is important to verify whether IFNβ treatment could have any effect on the life cycle and activity of JCV and thus whether a synergic effect of IFNβ and natalizumab in triggering the two PML cases is a possibility. IFNβ is well known for its antiviral activity, but it has also been shown to have an immunosuppressive effect, leading to the increase of cytokine expressions and of the risk of infections (Rudick *et al*, 1998).

The results of our study indicated a significantly lower presence of JCV DNA in the blood of the IFNβ-treated MS patients compared to the untreated patients and the healthy controls. These findings suggest that IFNβ can have an antiviral effect on JCV, as already observed for Human herpesvirus 6 (HHV6), which has been reported to be less frequent in the blood of INFβ-treated relapsing-remitting MS patients (Hong *et al*, 2002; Alvarez-Lafuentes *et al*, 2004).

Based on our results, the role of INFβ in the risk of developing PML could be probably ruled out, although periodic assessments of blood samples from relapsing-remitting MS patients for the presence and the active replication of JCV could offer helpful information serving to define interrelations between MS treatment and the life cycle of the virus.

Material and methods

Subjects

Ninety-eight patients (57 females and 41 males; mean age: 36 years; age range: 19–94 years) with clinically defined relapsing-remitting MS, were recruited from the Neurology Department of the Don C. Gnocchi Foundation, ONLUS, IRCCS. Of these patients, 59 had been undergoing treatment with INFβ (Avonex and Betaferon) for over 1 year and 39 were not

undergoing treatment as of at least 3 months prior to collection of the blood samples.

Ninety-eight HCs (58 females and 40 males), matched for sex and age, were also enrolled in the study. None of these subjects had clinical symptoms indicating viral infections at the time of blood sample collection. All participants provided their informed consent prior to enrollment.

DNA extraction and JCV Q-PCR

Peripheral blood was collected from all MS patients and HCs; DNA was isolated using phenol-chloroform extraction, according to standard procedures.

DNA was analyzed using Q-PCR for the detection and quantification of the JCV genome, following a previously described protocol (Delbue *et al*, 2005). Briefly, 250 ng of DNA were used as template in each reaction and a 54-bp amplicon in the JCV large T antigen region was detected. Each sample was analyzed in triplicate: samples that did not yield three positive reactions were repeated in triplicate and viral load results were given by the mean of the three positive reactions. Each run contained a negative control, constituted by the reaction mixture without the DNA template. A positive control consisted of serial dilution of the plasmid containing the entire JCV genome. The detection limit was 2 copies/μl, which is equivalent to 40 copies/μg of DNA extracted from blood.

Statistical analysis

Statistical analyses were carried out using the odds ratio (OR), calculated by Woolf's method, with the respective 95% confidence interval, and performing the chi-squared test for small samples with Yates' continuity correction. A P value of less than .05 was considered to be statistically significant. All tests were two-sided.

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