Published in final edited form as:

J Neurovirol. 2008 October ; 14(5): 455–458. doi:10.1080/13550280802356837.

Rearrangement of the JCV regulatory region sequence in the bone marrow of a patient with Rheumatoid Arthritis and Progressive Multifocal Leukoencephalopathy

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Abstract

The polyomavirus JC (JCV) is the etiologic agent of Progressive Multifocal Leukoencephalopathy (PML). JCV remains quiescent in kidneys, where it displays a stable archetypal regulatory region (RR). Conversely, rearranged JCV RR, including tandem repeat patterns found in the CNS of PML patients, have been associated with neurovirulence. The precise site and mechanism of JCV RR transformation is unknown. We present herein a patient with Rheumatoid Arthritis treated with methotrexate, who developed PML and had a rapid fatal outcome. JCV DNA PCR was positive in CSF, bone marrow, blood and urine. Double immunohistochemistry staining demonstrated that 9% of bone marrow CD138⁺ plasma-cells sustained productive infection by JCV, accounting for 94% of JCV-infected cells. JCV RR analysis revealed archetype and rearranged RR forms in bone marrow, while RR with tandem repeat was predominant in blood. These results suggest that the bone marrow may be a potential site of JCV pathogenic transformation. Further studies will be needed to determine the prevalence of JCV in bone marrow of immunosuppressed individuals at risk of PML and characterize the RR and phenotype of these JCV isolates.

Keywords

JC virus; Bone marrow; Rheumatoid Arthritis; Progressive Multifocal Leukoencephalopathy

Introduction

Progressive multifocal leukoencephalopathy (PML), (Koralnik, 2006) caused by JC virus (JCV) is a fatal demyelinating disease of the brain which occurs in up to 5% of patients with AIDS and 3% of patients with lymphoproliferative disorders treated with antineoplastic purine analogs. JCV remains quiescent in the kidneys and can be found in urine of 30% healthy and

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Nucleotide sequence accession numbers: The GenBank accession numbers for the sequences determined in this study are as follows, in order of appearance in Fig. 2A, item 4: a, plasma EU747351; b, urine EU747352; c, bone marrow EU747353; d, bone marrow EU747354.

immunosuppressed individuals alike. Although profound cellular immunosuppression is necessary for the development of PML, the site of JCV reactivation is unknown. Moreover, while the JCV regulatory region (RR) found in urine samples has a stable structure, called the “archetype”, isolates from the CSF and brain of PML patients usually contain duplications and deletions, which have been associated with neurotropism and neurovirulence (Jensen and Major, 2001). JCV has been found in the bone marrow (BM) in a dozen of individuals, from PML patients (Houff *et al* 1988), leukemia patients (Schneider and Dorries, 1993), or bone marrow transplant recipients (Coppo *et al*, 1999).

We analyzed JCV RR sequences in the bone marrow, blood and urine samples of an HIV-negative patient with PML and rheumatoid arthritis.

Materials and Methods

DNA from bone marrow, PBMC, plasma and urine samples was extracted and samples were tested by using the Real Time PCR technology as previously described (Lima *et al*, 2007). We then performed a nested-PCR that amplified a fragment of 353 bp of JCV RR (Ferrante *et al*, 2003) followed by cloning and sequencing of positive samples.

Double immunohistochemical staining (IHC) was performed on formalin fixed, paraffin-embedded BM biopsy tissue sections. The anti VP1 antibody PAB597 (a generous gift from Dr Walter Atwood), was used to stain for VP1 while the anti CD138 ab (clone MI15, Dako, Carpinteria, CA) was used to stain plasma cells.

Analysis of JCV-specific CD8⁺ cytotoxic T lymphocytes (CTL) was performed in blood samples by tetramer staining assay as described elsewhere (Lima *et al*, 2007).

Case report

A 70 year old HLA A*0201⁺ man with history of rheumatoid arthritis (RA) treated with methotrexate 20 mg qw, chloroquine 500 mg 4qw, and leucovorin for three years, presented with a right sided hemiparesis and confusion.

MRI demonstrated an area of hyperintense signal on T2 and FLAIR images in the left temporo-parietal subcortical region, as well as scattered small areas of hyperintensity in the periventricular white matter. A follow up MRI showed progression of the lesions in the left temporal-parietal region (Figure 1). Methotrexate was discontinued because of presumptive diagnosis of PML.

There were normal T-lymphocyte counts (CD4⁺ 855/mm³, CD8⁺ 522/mm³, CD4/CD8 ratio 1.6). The diagnosis of PML was established by detection of JCV DNA in the CSF by PCR. A minute quantity of JCV-specific CD8⁺ CTL response was detected in the blood using the tetramer staining assay but these cells were not present anymore upon repeated analysis one month later. The patient was treated with cytarabine 2mg/kg/day iv for 5 days and mirtazapine 15 mg a day (Elphick *et al*, 2004) but he died 13 weeks after the first neurological symptoms.

The JCV viral load was 2.2×10^3 copies/ml plasma, 9.4×10^1 copies/microgram PBMC DNA, and 6.24×10^3 copies/ml urine. Results of JCV RR sequence analysis are shown in Figure 2. All JCV RR clones from plasma had a tandem repeat of 98bp elements consistent with the neurotropic Mad-1 RR, and urine samples contained an RR with partial truncation and duplications of the 98 bp element, an intact 23 bp insert and partial truncations of the 66bp insert (Fig. 2 A). Interestingly, the major form of bone marrow RR (92% of the clones) contained a partial duplication of the 98bp element and truncation of the 23 and 66bp inserts, while a minor BM species of RR was similar to archetype (8% of the clones).

These results indicate that archetype JCV RR is present in BM. Furthermore the additional presence of a major rearranged clone suggests that the marrow may be a potential site of rearrangement, leading to its neutrotropic phenotype and subsequent hematogeneous CNS spread. Unfortunately CSF was not available for JCV sequence analysis and an autopsy was not performed. Finally, although archetype JCV RR is most commonly seen in urine of subjects, with or without PML (Jensen and Major, 2001), these results suggest that RR with partial tandem repeat can also arise from the kidney.

Double immunohistochemical staining (IHC) performed on BM biopsy tissue sections using an anti-JCV VP1 and anti-CD138 ab showed that 94% of JCV-infected cells were CD138⁺ plasma cells, and these accounted for 9% (171/1919) of all plasma cells present in an entire section of the biopsy specimen (Fig 2B).

Discussion

Our results confirm and expand a previous report showing the presence of JCV in mononuclear BM cells (Houff *et al*, 1988), and other studies showing association of JCV with B lymphocytes in peripheral blood (Monaco *et al*, 1996). However, JCV was also present in CD138-negative cells in the BM of the present patient, which we could not characterize further because of the limited amount of tissue available (Figure 2 B, arrow). These results are consistent with our earlier observation, which indicated that JCV can be found in association with multiple peripheral blood leukocyte subpopulations (Koralnik *et al*, 1999). Furthermore, our findings suggest that the BM is a compartment where the stable archetype JCV RR may acquire rearrangements associated with neurotropism and neurovirulence, as postulated in PML caused by the immunomodulatory medication natalizumab (Ransohoff, 2005).

Nevertheless we cannot exclude that rearrangement of JCV could have occurred elsewhere, such as in tonsillar tissue, where both archetype and rearranged regulatory regions have been found, before the virus reached the bone marrow (Kato *et al* 2004, Monaco *et al* 1998).

Only few cases of PML have been described in patients with RA who were treated with chlorambucil (Calabrese *et al*, 2007), or corticosteroids (Koralnik *et al*, 1995). It is likely that the immunosuppressive treatment, rather than RA, caused JCV reactivation and PML. The present patient was treated with methotrexate, he had normal T-lymphocyte counts, and only a minute, transient CTL response against JCV concomitant to an inflammatory reaction in PML lesions (Figure 1). Although such MRI finding has been associated with a longer survival in PML, his immune response was weak, unable to prevent disease progression and a fatal outcome.

Another case of methotrexate-associated PML has been reported in a patient with systemic lupus erythematosus. PML should therefore be differentiated from a unique type of necrotizing leukoencephalopathy (Oka *et al*, 2003) and from reversible posterior leukoencephalopathy (Abali *et al*, 2005) that may rarely be toxic manifestations of methotrexate therapy.

This is the first report of a detailed analysis of JCV RR from BM of a PML patient and suggests that rearrangements in the RR commonly associated with neurovirulence and PML may occur in this compartment.

Acknowledgements

This work was made possible in part by Public Health Service Grants R01 NS/AI 041198 and NS 047029 to IJK.

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Fig. 1. Brain MRI shows multiple PML lesions

A Fluid attenuation inversion recovery (FLAIR) MRI image shows hyperintense progressive multifocal leukoencephalopathy lesions in the white matter of both parietal lobes, devoid of swelling or mass effect (arrows, panel A). Lesions appear hypointense in T1-weighted images, and a faint peripheral enhancement is seen after administration of gadolinium (arrowheads, panel B)

Fig. 2.

Fig. 2A: **Sequencing results of JCV RR in plasma, urine and bone marrow**

1) Representation of the JCV RR. The NF-1 binding sites are indicated. Each 98 bp unit is indicated by an open box (nt 12–109 and 110–207). Core indicates origin of replication.

2) Neurotropic JCV Mad-1 RR

3) The archetype (kidney type) contains one 98 bp unit with one 23 bp insert (gray box) at position 36 and one 66 bp insert (black box) at position 92. Dark lines following the elements represent the late promoter. Deletions are marked by dotted lines.

4) The patient's RR from plasma (a), urine (b), and bone marrow (c+d) are displayed. Nucleotide positions correspond to the Mad-1 sequence. Adenine at nucleotides 85 and 183 in

Mad-1 is replaced by guanine in other sequences. The asterisk indicate NF-1 binding sites, associated with enhanced viral replication.

Fig 2 B: Localization of JCV-infected cells in bone marrow by double immunostaining for JCV VP1 protein and plasma cell marker CD138

A JCV-infected plasma cell (*) stained positively for both VP1 protein (brown chromogen, nuclear staining), and plasma cell marker CD 138 (purple chromogen, membranous staining). Uninfected plasma cells (purple chromogen, membranous staining, arrow heads) and one JCV-infected non-plasma cell (brown chromogen, nuclear staining arrow) can also be seen. The presence of the VP1 capsid protein indicates a full replicative cycle of the virus in these cells (Bar = 25µm).