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Case Report

Rearrangement of the JC virus regulatory region sequence in the bone marrow of a patient with rheumatoid arthritis and progressive multifocal leukoencephalopathy

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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 central nervous system (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 polymerase chain reaction (PCR) was positive in cerebrospinal fluid (CSF), bone marrow, blood, and urine. Double-immunohistochemical 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, whereas 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. *Journal of NeuroVirology* (2008) **14,** 455–458.

Keywords: JC virus; bone marrow; rheumatoid arthritis; progressive multifocal leukoencepalopathy

Introduction

Progressive multifocal leukoencephalopathy (PML) (Koralnik, 2006), caused by JC virus (JCV), is a fatal demyelinating disease of the brain that occurs in up to 5% of patients with acquired immunodeficiency syndrome (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 immunosuppressed individuals alike. Although profound cellular immunosuppression is necessary for the development of PML, the site of JCV reactivation is unknown. Moreover, whereas the JCV regulatory region (RR) found in urine samples has a stable structure, called the ''archetype,'' isolates from the cerebrospinal fluid (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 individuals, from PML patients (Houff *et al* 1988), leukemia patients (Schneider and

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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 a human immunodeficiency virus (HIV)-negative patient with PML and rheumatoid arthritis.

Case report

A 70-year-old HLA A*0201$^+$ man with history of rheumatoid arthritis (RA) treated with methotrexate 20 mg per week, chloroquine 500 mg four times per week, and leucovorin for 3 years presented with a right-sided hemiparesis and confusion.

Magnetic resonance imaging (MRI) demonstrated an area of hyperintense signal on T2 and FLAIR images in the left temporoparietal 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/mm^3, CD8$^+$ 522/mm^3, 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$^+$ cytotoxic T-lymphocyte (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 2 mg/kg/day intravenously (i.v.) 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/μg peripheral blood mononuclear cell (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 98-bp 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 66-bp insert (Figure 2A). Interestingly, the major form of bone marrow RR (92% of the clones) contained a partial duplication of the 98-bp element and truncation of the 23- and 66-bp inserts, whereas 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 central nervous system (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.

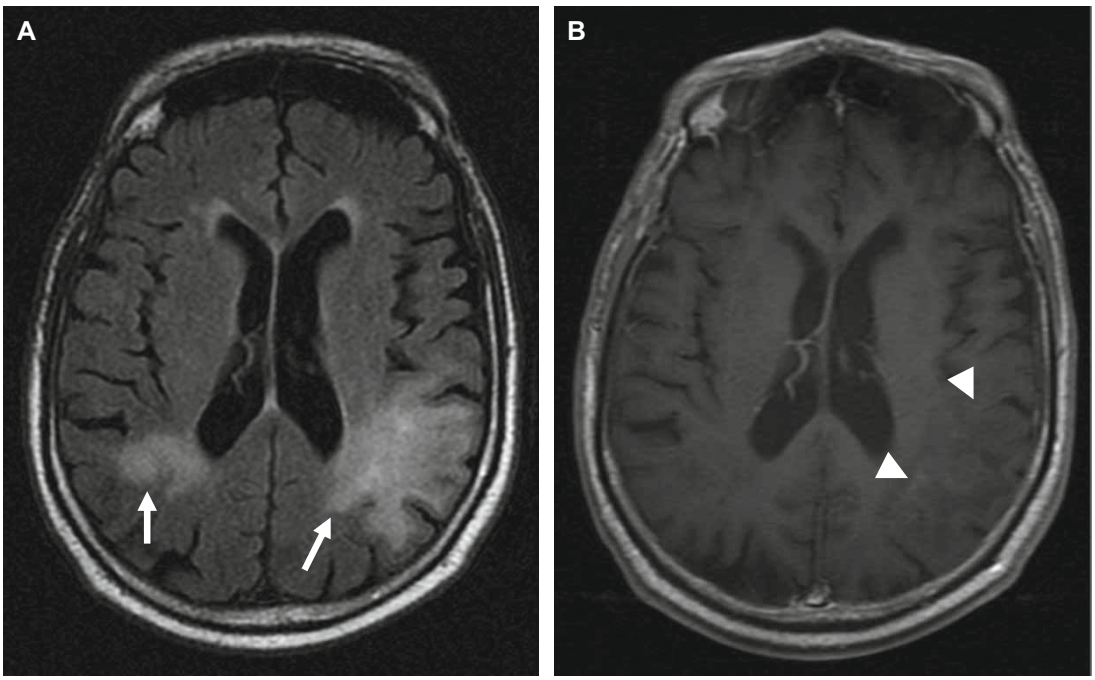

Figure 1 Brain MRI shows multiple PML lesions. A fluid attenuation inversion recovery (FLAIR) MRI image shows hyperintense progressive multifocal leukoencephalopathy lesions in the white matter of both parietal lobes, devoid of swelling or mass effect (*arrows,* **A**). Lesions appear hypointense in T1-weighted images, and a faint peripheral enhancement is seen after administration of gadolinium (*arrowheads,* **B**)

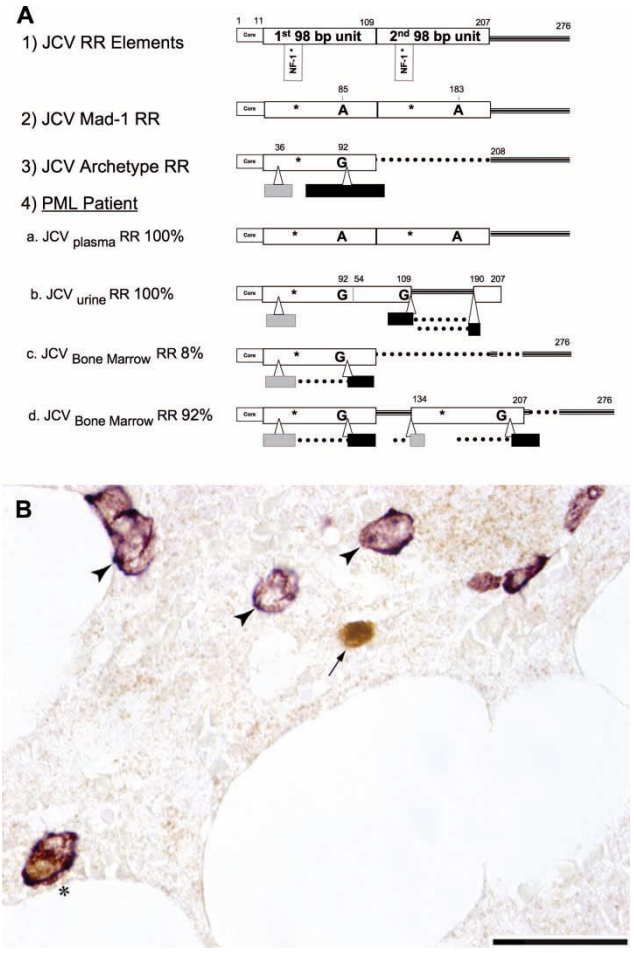

Figure 2 (**A**) Sequencing results of JCV RR in plasma, urine, and bone marrow. 1) Representation of the JCV RR. The NF-1 binding sites are indicated. Each 98-bp unit is indicated by an open box (nucleotides 12 to 109 and 110 to 207). Core indicates origin of replication. 2) Neurotropic JCV Mad-1 RR. 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. (**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; *arrowheads*) and one JCV-infected nonplasma 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 = 25 μm).

Double-immunohistochemical staining (IHC) performed on BM biopsy tissue sections using anti-JCV VP1 and anti-CD138 antibodies 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 (Figure 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 2B, *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 tonsilar 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 rheumatoid arthritis (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 erythematous. 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), which on rare occasions may 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.

Materials and methods

DNA from bone marrow, peripheral blood mononuclear cell (PBMC), plasma, and urine samples was

extracted and samples were tested using real-time polymerase chain reaction (PCR) as previously described (Lima *et al*, 2007). We then performed a nested PCR that amplified a 353-bp fragment 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, whereas the anti-CD138 antibody (clone MI15; Dako, Carpinteria, CA) was used to stain plasma cells.

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

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

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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