JC virus variant associated with cerebellar atrophy in a patient with AIDS

Short title: JC virus variant in cerebellar atrophy

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Abstract.

The human polyomavirus JC (JCV) is the agent of progressive multifocal leukoencephalopathy (PML). It has also been recently involved in cerebellar atrophy. Factors involved in this entity are elusive. We present a case of a human immunodeficiency virus-infected patient with PML and cerebellar atrophy. In addition to a compartmentalization of JCV strains between urine, cerebrospinal fluid and cerebellum, specific rearrangements in the JCV regulatory region were observed in cerebellum resulting in alterations of transcription factor binding sites. Our data underline the importance of searching JCV in HIV infected patients with cerebellar disorders and suggest that mutations in the regulatory region may be involved in cerebellar degeneration.

Key Words.

Polyomavirus JC; cerebellar atrophy; granule cell neuronopathy; AIDS; regulatory region.
Introduction.

Cerebellar disorders are uncommon among patients infected with Human Immunodeficiency Virus (HIV) and include various etiologies (2). The polyomavirus JC (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), with lesions usually restricted to the white matter. In few cases, PML has also been associated with alterations in the grey matter. JCV has recently been involved in a neuronal infection restricted to the granule layer of cerebellum (3, 9, 11) and is now considered as the etiology of a new entity named JC virus granule cell neuronopathy (GCN). The factors involved in the development of JCV associated neuronal disorders are poorly understood. In patients with PML, sequence variations in the JC virus capsid protein VP1 and in the regulatory region (RR) which regulates early and late viral genes expression have been shown to occur in cerebral JCV isolates (7, 17-19) (14).

Viral characteristics associated with JCV cerebellar disorders remain to be explored. We describe here the case of an HIV-infected patient presenting a PML and cortical atrophy of the cerebellum associated with JCV infection. We present data of genetic rearrangements occurring in the regulatory region of the specific cerebellum JCV isolate which result in transcription factor binding sites alterations.

Case description.

A 38-year-old HIV-positive man was admitted on June 27th 2008 for subacute onset of a cerebellar disorder. HIV-1 infection was diagnosed two years earlier with cachexia due to disseminated *Mycobacterium avium* infection. His medical history included several episodes of CMV reactivation, oropharyngeal candidiasis and anal condyloma. He had no history of alcohol intake. Since the initiation of highly active antiretroviral therapy (HAART) (lopinavir, ritonavir, emtricitabine and tenofovir) in 2007, plasma HIV-1 RNA has remained undetectable for 8 months but his absolute CD4+ lymphocyte count has always remained
below 50/mm$^3$ with uncontrolled mycobacterial intra-abdominal infection. 

On June 27$^{th}$, the clinical examination showed evidence of a pancerebellar syndrome including severe ataxia, kinetic tremor, central nystagmus and major dysarthria. The patient was unable to stand unassisted. Muscle stretch reflexes were brisk and plantar responses were flexor bilaterally. Sensory examination was normal. Brain Magnetic Resonance Imaging (MRI) revealed mild, generalized cerebral atrophy with prominent cerebellar atrophy involving midline and hemispheric structures, with enlarged fourth ventricle (Figure 1). FLAIR Sequence revealed few white matter abnormalities. No focal abnormalities were observed. His absolute CD4+ lymphocyte count was 39/mm$^3$ and plasma HIV-1 RNA level was < 50 copies/ml. Molecular analysis for viral etiologies showed in cerebrospinal fluid (CSF) the absence of HIV, cytomegalovirus, varicella-zoster virus, herpes simplex virus type 1 and type 2, Epstein-Barr virus, human herpes virus 6 and BK virus along with absence of syphilis antibodies. In contrast, JCV DNA detection was positive in CSF by a real-time PCR assay targeting the large T antigen (6). In CSF, neuronal auto-antibodies were negative. Vitamin plasma levels were within the normal range. JCV DNA was also detected in urine. Quantification of JCV DNA, performed in CSF (27/08/08), in urine (02/10/08) and in postmortem cerebellum biopsy (14/10/08) were 4.7, 6.80 and 8.08 log$_{10}$ copies per 150 000 cells, respectively. Despite the continuation of HAART, his CD4+ lymphocyte count remained unchanged and the patient clinical status progressively worsened with cachexia, major asthenia and confusion. He died a few months later.

**Methods**

**Neuropathologic examination.** Autopsy was obtained according to current rules, including interrogation of the National Register (Agence de la Biomédecine) and information given to next of kin. After fixation, sampling, paraffin embedding and standard histology techniques,
immunohistochemistry of polyomavirus was performed on cerebellar slides using a rabbit polyclonal antibody to large T antigen, provided by Dr DL Walker, University of Wisconsin Medical School, Madison (dilution 1/500 overnight). A double labeling was performed using a monoclonal mouse anti-human, 2F11 clone, Dako®, in a Ventana® automat associated with the antipolyomavirus antibody (figure 2E). Detection was made with Ventana® Ultraview DAB for neurofilaments and Dako® Kit REAL detection system for polyomavirus.

**Analysis of VP1 and RR sequences.** Nucleic acids were extracted from urine, CSF and cerebellum samples using the EasyMag System (Biomérieux) and used to amplify the VP1 and RR gene as previously described (1, 13, 18). Purified PCR products were sequenced using an ABI 3100 Genetic Analyser (Applied Biosystems). Sequences were aligned using Sequence Navigator software version 1.0.1 (Applied Biosystems). This approach enables the detection of predominant variants in each site with a 20% limit of detection for minor variants.

**Results**

Macroscopic examination of the cerebellum showed severe atrophy with dramatic cortical thinning, in both the vermis and cerebellar hemispheres. Microscopically, there was a loss of granular neurons throughout the cerebellum with sparing of the Purkinje and the molecular layers. Moreover, the white matter presented severe lesions, with enlarged bizarre astrocytes and enlarged oligodendrocyte nuclei. Immunohistochemistry stains for polyomavirus proved the diagnosis of PML with nuclear positivity of astrocytes and oligodendrocytes (Figure 2). Co-staining of JCV and neuronal cells found no evidence of infected granule cell neurons (Fig 2E), but showed a juxtaposition of infected JCV cells and neurofilament positive cells, in the context of an extensive destruction of the granule cell layer. This observation favors cytotoxicity from satellite oligodendrocytes rather than direct infection of granule cell
neurons, although this latter hypothesis cannot be ruled out due to the extent of the damage.

Sequence variations in VP1 and RR from urine, CSF and cerebellum samples were analyzed to look at the compartmentalization of the virus and to assess a possible strain specificity associated with cerebellar atrophy. Phylogenetic analysis based on VP1 sequences indicated that the strain was related to genotype 5 while the RR sequence was closely related to 1A genotype (data not shown).

Considering the region of VP1 involved in cell entry, strains from three compartments were similar, except a substitution of serine-123 with cysteine in the outer DE loop of the cerebellar strain. Such mutation has been observed previously in PML cases but not known to confer new phenotypic characteristics (18). A deletion associated with a frame shift in the C terminal sequence of the VP1 gene has also been previously observed in a patient with cerebellar atrophy and was hypothesized to be associated with modifications in viral post-entry events. In our case the C terminal region of VP1 showed no major difference with PML JC virus variants (1). Only two substitutions of methionine-339 with isoleucine and lysine-345 with arginine, which do not change amino acid properties, were present in all sequences.

The sequence analysis of RR showed three distinct strains in urine, CSF and cerebellum. The successive loss of genetic sequences suggests a genetic drift from urine to CSF and ultimately to cerebellum (Figure 3). The sequences in the cerebellum and CSF had a deletion of 22 bp compared to the sequences from urine and the sequence in the cerebellum had another additional 23 bp deletion. According to the previously described classification of RR forms (8), the RR from urine was of type II-S with a single 98-base-pair (bp) unit and one 23-bp insert (Block B) and one 66-bp insert (Block D), as seen in the JCV archetype. The cerebellum and CSF RRs had a similar form but had one deletion of 22 bp in the block D. The cerebellar sequence had an additional 23 bp deletion corresponding to the block B, 7bp
downstream of the TATA box (nt37-nt59) as the JCV Mad1 reference strain (4), and encompassing the GA box which is the binding site of the Sp1 transcription factor. Interestingly, this deletion has resulted in a new motif (AGGGAAGGGA) called lytic control element (LCE) (12) creating a binding site for Pur alpha and YB-1. In addition two other mutations at nucleotide numbers 71 (G>T) (Archetype numbering) and 76 (G>T) disrupted the binding sites for NF-1 and GF-1 respectively (15). The binding site for AP-1 was maintained. The three isolates showed absence of tandem repeat of the 98 bp element, usually seen in JCV isolated from the central nervous system of PML patients.

Discussion

Our patient experienced extensive cerebellar atrophy involving the granule cell layer which was concomitant with the development of a PML. Except JCV infection, no other etiology was found to explain cerebellar disorders. The high cerebellum viral load, the unique pattern of genetic mutations in RR and the JCV immunostaining on cerebellar biopsy, suggested that JCV was associated with a granule cell neuronopathy. JCV is known to productively infect astrocytes and oligodendrocytes resulting in demyelinated white matter. Cerebellar atrophy, which implies neuronal lesions, is absent in typical PML. It has been recently shown among HIV infected patients that latent JCV infection of cerebellar granule cell neurons (GCNs) was actually frequent (16). The mechanisms leading to JCV lytic replication of GCNs and then to cerebellar atrophy are not determined.

Amino acid substitutions (55, 60, 265, 267, 269) on the surface of VP1 capsid protein associated with typical PML (14) or deletion in the C-terminal portion of VP1 described in a patient with PML and GCN (1) were not found in this case. In contrast, in the present case the few substitutions observed in the cerebellar strain do not support a specific phenotype due to altered VP1 protein. The RR sequence analysis showed a compartmentalization of JCV and
suggested a progressive genetic drift from urine to CSF and finally to cerebellum. RRs from
CSF and cerebellum had partial deletion of the block D, as some other RR sequences from
HIV infected with PML that have been associated with increased early gene expression and
higher JCV replication in vitro (5) which is consistent with the high viral load observed in
cerebellum for this patient. In contrast to previous results from patients suffering from JCV
granule cell neuronopathy, the cerebellum strain showed a specific deletion in the RR region
encompassing the binding site of Sp1, known to be a strong inducer of JCV expression in
glial cells. The deletion has resulted in the creation of a binding site for Pur alpha and YB-1.
Although these two factors also have the ability to interact with the 23-bp sequence element
deleted in the cerebellar isolate, it has been shown that their interaction with the LCE had
more effect on early and late gene transcription (12). Notability, it has been shown in vivo that
block B deletion is associated with higher cerebrospinal fluid JCV DNA level (10). Except in
Mad1 strain, this deletion has been previously reported only in one patient with PML, with no
evidence of cerebellar atrophy, but a pseudobulbar paralysis (19). In addition two other
mutations in cerebellar sequence abolished two binding sites for transcription factors,
including the binding site for NF-1, considered as a major transcription factor of JCV.
Although in vitro studies have demonstrated that the number of NF-1 binding sites is
proportional to the level of JCV transcription in glial cell lines, an in vivo analysis including
45 patients has in contrast shown that the number of NF-1 binding sites is negatively
correlated with JCV viral load (10). The sequencing results without excluding the presence of
minor variants clearly demonstrates that the majority of cerebellar strains harbour mutations
in the regulatory region not observed or underrepresented in other compartments. The specific
effects of such rearrangements on cerebellar pathogenesis and GCNs infection deserve further
investigation. In conclusion, our observation underlines the importance of ascertaining
whether JCV is present in HIV infected patients with cerebellar disorders even when gray
matter is mainly affected and shows that mutations in the regulatory region may be involved in cerebellar atrophy.

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Conflict of interest. The authors do not have any conflict of interest to declare.
References


Legends

**Figure 1. Cerebellar magnetic resonance imaging (MRI).** Axial no contrast T1-weighted (A) and coronal contrast-enhanced T1-weighted MR (B) images show atrophy of the vermis and cerebellar hemispheres with enlargement of the 4th ventricle.

**Figure 2. Polyomavirus JC infection of cerebellar granule cell layer.** A. Cerebellum, (hematoxylin eosin X 20). Severe alteration of the white matter (*) associated with dramatic atrophy of the granular layer (arrow). B. Same field as A at higher magnification (hematoxylin eosin X 100). Purkinje cells are still recognizable (arrow), contrasting with the dramatic loss of granule cells (*). In the deepest part of the granular layer, some enlarged cells are reminiscent of modified oligodendrocytes (arrow head). C. Same field as A (Polyomavirus immunohistochemistry X 20). The dot like labeling is predominant in the white matter. D. Same filed as A & B (Polyomavirus immunohistochemistry X 400). The enlarged nuclei of modified oligodendrocytes are labeled with the anti-Polyomavirus antibody. E. Co-immunostaining of polymavirus and neurofilaments (X 400). Polyomavirus infected cells are stained in red (*) and neurofilaments in brown (arrow head).

**Figure 3. Sequencing results of JCV Regulatory Region (RR) between strains detected in urine (MBuri), cerebrospinal fluid (MBcsf) and cerebellum (MBcer).** The nucleotide numbers indicated on top are based on the archetype sequence (8). The regulatory region sequence section contains the origin of DNA replication (ORI) followed by sequence sections designated A, B, C, D, E and F in italic. The base-pair length of each section is noted in italic (8). The TATA box and the GA box are represented respectively by TATA and GA in grey boxes. The start of Agnogene is indicated by an arrow labelled “Agno”. Dotted lines represent deletions or regions not present. The representation of MAD-1 sequence (MAD-1) is shown at
the bottom. The location of the 98 bp unit repeat insert (including A, C and E sections) is indicated by a triangle. Single letters in bold indicate nucleotides.