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Gone over to the dark side: natalizumab-associated JC virus infection of neurons in cerebellar gray matter

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The case report of natalizumab-associated JC virus granule cell neuronopathy (JCV GCN) by Schippling et al. in this issue of the Annals[1] will come as a surprise for many. It shouldn't. Thirty years ago, E.P Richardson reported areas of cell loss in the cerebellar granule cell layer in 5% of cases of progressive multifocal leukoencephalopathy (PML)[2]. Twenty years later, we observed a patient with AIDS who developed bilateral subcortical frontal lobe lesions of PML as well as unexplained cerebellar atrophy and dysfunction. Neither MRI nor post mortem analysis showed any demyelinating lesions in the cerebellar white matter, but immunohistochemistry (IHC) staining revealed JCV infection of granule cell neurons in the periphery of areas of focal cell loss in the cerebellar granule cell layer. Laser capture microdissection of infected cells confirmed the presence of JC virus and mature viral particles were detected in the nuclei of granule cell neurons by electron microscopy [3]. Comparison of the full sequence of the viral strains found in this patient's cerebral white matter and the cerebellar gray matter showed that the neuronal isolate had a unique 10 nt deletion in the C-terminus of the VP1 gene[4]. These findings thereby shattered two long enduring tenets of JCV biology, demonstrating that JCV does not only infects glial cells, and that mutations of JCV coding region may be associated with its pathogenicity, and not merely with the patient's geographic origin[5].

Soon thereafter, we described an HIV-infected patient who developed a chronic cerebellar syndrome with marked atrophy despite a slow but steady rise in $CD4^+$ T-cell count on combined antiretroviral therapy (cART). JCV infection was restricted to granule cell neurons and hence this novel entity, distinct from PML, was named JCV granule cell neuronopathy (JCV GCN)[6]. Subsequent cases were reported in the Americas[7-13], Asia[14], Australia[15-16], and Europe[17], mostly in HIV-infected patients or immunosuppressed individuals, with or without concomitant PML. Molecular analyses of brain or CSF samples from 6 of these JCV GCN patients showed identical or similar mutations in the VP1 gene C-terminus[11] (Figure), a feature otherwise absent from more than 569 JCV sequences deposited in GenBank, precluding a chance association.

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How could these minimal sequence alterations be associated with a radical switch in cellular tropism of the virus? The viral capsid is composed of 72 pentamers of the VP1 protein, which are hooked together by the very area that contains these mutations. Therefore, the mutated areas are not exposed on the surface of the capsid, and are not likely to directly alter binding to cellular receptors. However, they may be associated with the structural integrity of the virions, and may potentially modulate post entry events including uncoating, transport and replication of JCV DNA, protein expression and assembly of the viral capsid, which could then enable productive infection of granule cell neurons. In vitro modeling showed that the GCN1 mutant was replication-competent, remained stable overtime, and had a disadvantage for growth in glial cells compared to undeleted JCV strain[11].

Although JCV GCN remains far less frequent than PML, the prevalence of JCV infection of cerebellar granule cell neurons is likely underestimated. Indeed, retrospective analysis of archival brain samples showed that up to 51% HIV-seropositive PML patients and 3% HIV-seropositive individuals without PML had demonstrable JCV infection of these neurons by IHC, regardless whether classic PML lesions were also present in the nearby cerebellar white matter [18]. This may explain why mutated JCV strains can be found concomitantly to undeleted strains in CSF and blood of PML patients[11] suggesting that the GCN-type mutations may arise outside of the CNS.

Ten years after the initial description of JCV infection of neurons, JCV GCN has come of age. Furthermore, the case report by Schippling et al represents 2 new milestones: Indeed, this is the first time JCV GCN has been associated with natalizumab and the first incidence of an immune reconstitution inflammatory syndrome (IRIS). The fact that JCV GCN also occurs in natalizumab-treated MS patients should not be a surprise. As of 6/4/2013, there has been 372 reported cases of PML associated with natalizumab therapy worldwide[19], and no genetic predisposition has been identified. The risk of PML is higher in patients treated with immunosuppressive medications prior to natalizumab[20], but this was not as factor here. However, PML incidence increases with duration of natalizumab treatment. Therefore, it may be significant that the symptoms of cerebellar dysfunction attributable to JCV GCN started only after four years of natalizumab monotherapy, and hence a long exposure to the medication may be necessary for the development of this condition.

More interesting is the occurrence of IRIS in this case. IRIS, characterized by clinical worsening, occasional contrast enhancement on MRI and inflammatory infiltrates on brain biopsy almost always occur in natalizumab-associated PML, after discontinuation of natalizumab therapy and plasma exchange[21]. Since JCV-infected glial cells express MHC Class I and II molecules on their surface, they are recognized and ultimately, destroyed by the T lymphocytes returning into the brain parenchyma, triggering the inflammatory reaction. Whether neurons can in fact express MHC molecules, a characteristic that renders them visible to the immune system, has long been a matter of debate[22]. The present case report should dispel any remaining doubt that cerebellar granule cell neurons, when infected by JCV, can indeed be the target of $CD8^+$ T-cells. As it is the case in PML-IRIS, JCV GCN-IRIS may also be devoid of contrast enhancement on MRI.

Now that JCV GCN has joined PML -a white matter disease- among the untoward side effects of natalizumab therapy, clinicians and their patients should be aware that unexplained cerebellar dysfunction or atrophy may be caused by JCV infection of neurons in the cerebellar gray matter. Diagnosis should be ascertained by JCV PCR in the CSF. Since the sensitivity of this test is unknown in this setting, a cerebellar biopsy or discontinuation of natalizumab therapy and plasma exchange should be considered if the JCV PCR comes back negative. IRIS will then have to be differentiated from MS flares if patients worsen clinically after natalizumab removal. These are daunting prospects, which will certainly complicate further the clinical management of these challenging patients.

However, this unfortunate event also gives us a chance to learn from history. In fact, JCV GCN is not the only condition caused by JCV infection of neurons. Cerebral cortical pyramidal neurons are also susceptible targets either in isolation, causing a gray matter disease called JCV encephalopathy (JCVE)[23-24], or in association with PML[18], another "surprise" that had been predicated already more than 40 years ago[25]. Since gray matter involvement is frequent in MS[26-27], the development of cerebral cortical lesions and associated dysfunction, including seizures, occurring during natalizumab treatment should also raise the suspicion of JCV infection.

Paradoxically, the fact that natalizumab has now gone over to the "dark" side of the brain was also needed to put JCV GCN squarely in the spotlight, transforming it from side curiosity into a reality that has become impossible to ignore. Hopefully, this increased awareness will be instrumental in helping us define novel avenues of research to understand better all aspects of JCV pathogenesis, and finally challenge us to find a treatment for this cunning virus.

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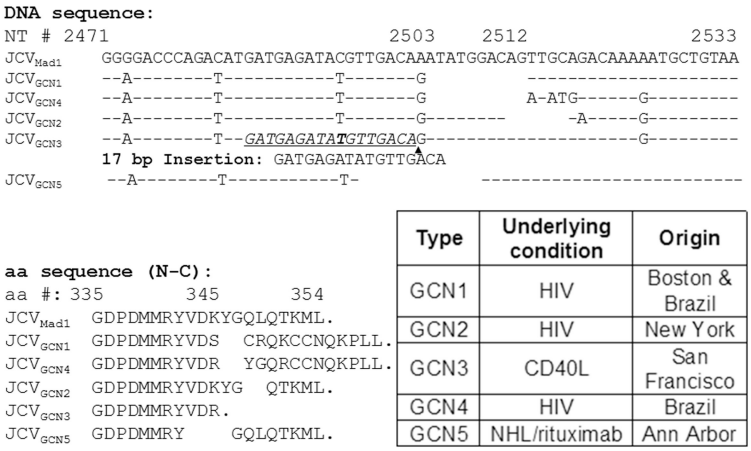

Type	Underlying condition	Origin
GCN1	HIV	Boston & Brazil
GCN2	HIV	New York
GCN3	CD40L	San Francisco
GCN4	HIV	Brazil
GCN5	NHL/rituximab	Ann Arbor

Figure 1.

Alignment of DNA and amino acid sequences of VP1 C terminus of all identified GCN-type JCV strains. The nucleotide and amino acid position numbers correspond to prototype JCV_{Mad1}. All GCN-type mutations (including both deletion and insertion) occur between nt 2496 and 2516. Dashed lines denote sequence homology. Line interruption denotes deletion. The sequence underlined and in italics in JCV_{GCN3} is the one that is duplicated in the 17bp insert at nt 2502. The underlying condition and geographic origin of the patients is indicated. CD40L: CD40 ligand deficiency. NHL: non-Hodgkin lymphoma.