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Title: Symptomatic Cerebrospinal Fluid HIV-1 Escape with no Resistance-associated Mutations Following Low-level Plasma Viremia

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Abstract: The majority of neurologically symptomatic cerebrospinal fluid HIV-1 escape cases are connected with resistance-associated mutations and potentially explained by low cerebrospinal fluid antiretroviral concentrations. However, there are still significant knowledge gaps regarding the physiopathology and long-term management of neurosymptomatic viral escape. We report a case of Parkinson-like syndrome following cerebrospinal fluid HIV-1 escape in a 40 years-old female patient with an history of persistent low-level plasma viremia under treatment. No resistance-associated mutations, high viral diversity (*env* deep sequencing), adequate pharmacokinetics, atypical CD3-CD14-CD4+CD5-CD2-/CD7-/ lymphocytes, low level Epstein-Barr virus replication and white matter autoimmune reactivity were observed in the cerebrospinal fluid. Antiretroviral regimen modification led to rapid clinical and radiological improvements. This case may increase the current uncertain knowledge on the origin of cerebrospinal fluid HIV-1 and illustrates the consequences of uncontrolled compartmental viral replication; it also highlights the relevance and persistence of immune activation and the possibility of various detrimental mechanisms underlying neurosymptomatic viral escape.

Keywords: HIV, Neuroimmunology, Virology, Viral escape, Antiretrovirals

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Introduction

Human Immunodeficiency Virus (HIV) has been associated with various neurological disorders (Ferretti et al. 2015). Despite the striking effectiveness of highly-active antiretroviral therapy (HAART), several cases of cerebrospinal fluid (CSF) viral escape (CVE) have been reported (Edén et al. 2010; Edén et al. 2016; Canestri et al. 2010; Peluso et al. 2012). CVE refers to cases where subjects have CSF HIV-RNA 0.5-1 Log₁₀ greater than the corresponding plasma HIV-RNA or still detectable despite plasma undetectability (Ferretti et al. 2015). This condition is found in 10-36% of treated HIV-positive patients (Edén et al 2010; Edén et al. 2016), and it has been associated with long history of infection, severe immune depletion and incomplete adherence to HAART (Edén et al. 2010; Edén et al. 2016; Nightingale et al. 2016; Peluso et al. 2012). Such circumstances, coupled with inadequate antiretrovirals' penetration, may favour the selection of resistance-associated mutations (RAMs) within the central nervous system (CNS) leading to CVE (Ferretti et al. 2015). Most CVE are asymptomatic, probably representing the CSF equivalent of plasma viral blips with debated clinical significance (Edén et al. 2016). Nevertheless, a few cases of neurosymptomatic CVE have also been reported (Canestri et al. 2010; Peluso et al. 2012), and the most common sign was cerebellar ataxia (Canestri et al. 2010). In almost all of these cases RAMs were observed (Canestri et al. 2010; Peluso et al. 2012), supporting the hypothesis that low CNS drug concentrations allows their selection and facilitates CVE. Such neurological syndromes can usually be reverted by modifying treatment including drugs with better CNS penetration and activity (Ferretti et al. 2015; Canestri et al. 2010; Peluso et al. 2012). However, there are still significant knowledge gaps regarding the physiopathology and long-term management of neurosymptomatic CVE. We here report a case of neurosymptomatic HIV-1 escape without CSF RAMs that underwent extensive diagnostic assesment.

The Case

A 40-years-old woman was diagnosed with HIV-1 infection in the 90s; she started zidovudine plus lamivudine and K70R and M184V were rapidly selected. In the following years, she reported poor adherence to HAART and she later discontinued treatment. In 2008 she presented with *Pneumocystis jirovecii* pneumonia (13 CD4+ T-cells/uL; plasma and CSF HIV-RNA 557351 and 2545 cp/mL). No RAMs were observed in either samples, viruses were R5-tropic and brain magnetic resonance (MRI) resulted normal. Maraviroc, atazanavir/ritonavir and tenofovir-disoproxil-fumarate were started. Six months later her CD4+ count was 579 cells/uL, while her virological response was suboptimal due to persistent low-level plasma viremia, despite self-reporting good adherence. Atazanavir/ritonavir was replaced by once-daily darunavir/ritonavir following the observation of N88S/G73S on protease gene. In 2015, she developed headache, dizziness, severe gait abnormalities and significant unintentional tremors, depicting a Parkinson-like syndrome. Neurological examination revealed lower-limb weakness, tremors at rest, cogwheel rigidity, slow parkinsonian gait and alterations in deep tendon reflexes. **Brain MRI was abnormal and consistent with findings usually observed in cerebral, cerebellar and brainstem regions of symptomatic CVE cases (Ferretti et al. 2015; Canestri et al. 2010; Peluso et al. 2012)** (Fig.1). CSF examination revealed mononuclear pleocytosis, elevated proteins and normal glucose. CSF-serum albumin ratio was within the age-adjusted normality suggesting no blood-brain barrier impairment, while CNS immune activation was significant (elevated CSF neopterin, IgG index and intrathecal IgG synthesis; Tab.1). CSF cytofluorimetry showed the presence of an expanded subpopulation of atypical CD3-CD14-CD4+CD5-CD2-/CD7-/ lymphocytes (2.9% of CSF lymphocytes; see Supplementary Fig.1). CSF cultures and examinations for opportunistic infections were negative, although CSF EBV-DNA was 82 cp/mL; CSF and plasma HIV-RNA were 7566 cp/mL and 90 copies/mL. CSF genotypic testing showed no RAMs. Interestingly, an indirect immunofluorescence assay on primate cerebellum sections evidenced the patient's CSF binding on white matter structures (in a CV2/CRMP5-like pattern) associated to a pericellular synaptic reactivity around Purkinje cells (see Supplementary Fig.2). No known antigens were identified by commercially

available immunoblot tests (Hu, Ma2, NMDA) and myelin oligodendrocyte glycoprotein antibodies were negative on serum and CSF. We also isolated CSF HIV-RNA and performed 300 base pair, paired-end Illumina MiSeq deep sequencing of the V1-V3 region of the HIV *env* using the Primer ID method (Zhou et al. 2015). The analysis revealed a diverse viral population that likely evolved within the CNS over many generations (Fig.2). Antiretrovirals CSF concentrations were detectable (Tab.1) and similar to what previously described (Best et al. 2012; Calcagno et al. 2015). Her regimen was changed to twice-daily darunavir/ritonavir, raltegravir and etravirine, taking into account plasma RAMs and CSF inhibitory quotients (Calcagno et al. 2015). Three months later full clinical recovery was observed; a significant improvement in brain MRI was also recorded (Fig.1). Virological and inflammatory biomarkers at the time of escape and 3-9 months later are reported in Tab.1.

Discussion

Neurosymptomatic CVE is characterized by the acute/subacute onset of new neurological signs in HAART-treated patients presenting with discordance between plasma and CSF HIV-RNA when no other clinical condition may explain such manifestations (Ferretti et al. 2015). Our patient had virtually all the risk factors for CVE (Edén et al. 2010; Edén et al. 2016; Nightingale et al. 2016; Peluso et al. 2012): a very low CD4+ nadir, a long history of infection and antiretroviral treatment, previous HAART interruption and recurrent low-level plasma viremia. Furthermore, the CSF results and the typical white matter hyperintensities on T2-weighted and FLAIR sequences were similar to previous symptomatic CVE (Ferretti et al. 2015; Canestri et al. 2010; Peluso et al. 2012). Nevertheless, while the majority of neurosymptomatic CVE are associated with RAMs (Ferretti et al. 2015; Nightingale et al, 2016; Canestri et al. 2010; Peluso et al. 2012), in our case we documented no CSF drug resistance. Such a pattern may be generated by antiretrovirals not reaching inhibitory levels throughout the brain tissues or having poor activity in infected target cells (Calcagno et al. 2015; Gray et al. 2013). Despite CSF concentrations of our patient were above the

in vitro inhibitory concentrations (Best et al. 2012; Calcagno et al. 2015), they are just surrogates of CNS antiretrovirals activity. Lacking CNS tissues pharmacodynamics/pharmacokinetic data, we cannot rule out that our patients' antiretrovirals had a reduced concentration/activity in some CNS HIV-infected cells (Gray et al. 2013). Moreover, the genetic diversity of the CSF HIV-1 population we observed indicates that it was produced by a large number of productively infected cells within CNS, but it was unclear whether it was due to ongoing viral replication during HAART or to persistent production within CNS without replication.

To our knowledge, no reported symptomatic CVE was investigated for immunoreactivity against CNS antigens. Rather than being the cause of neurological syndromes, this phenomenon is likely due to brain cells and blood-brain barrier damage in an inflammatory milieu since immune hyperactivation may indeed lead to such non-sense production of autoantibodies (Lackner et al. 2010). Compartmental immune activation is a striking feature of symptomatic CVE (Spudich 2016), and in our patient it persisted for months despite viral control (Tab.1). Nevertheless, similar auto-reactivity has also been found to correlate with CSF HIV-RNA and HIV-associated neurocognitive disorders in cases without prominent neurotoxic inflammation (Lackner et al. 2010), so that further studies are needed to assess the conceivable pathogenic role of such findings in HIV-related neurological disorders. Lastly, this is also the third case of neurosymptomatic CVE with concurrent low-level CSF EBV-DNA (Peluso et al. 2012). We do not know if the atypical CSF T-cell subpopulation we reported was related to such a replication, to the CVE itself or to some other hidden ongoing process; the subpopulation was no more observed after both HIV-RNA and EBV-DNA disappearance. It has been speculated that some cases of CVE may be secondary to other CNS infections or inflammatory process leading to an influx of HIV-infected leukocytes carrying the virus into CNS (Ferretti et al. 2015). From this point of view, CVE may also not be always the primary cause of neurological syndromes, but rather an accompanying epiphenomenon (Ferretti et al. 2015).

In conclusion, CVE is most easily explained by the use of regimens that reach inadequate levels in CNS areas where HIV replicates, that have poor activity in CNS cells and/or by the presence of drug-resistant viruses within CNS. Symptomatic CVE was reversible once HAART was improved. This observation coupled with the absence of RAMs and the diversity of the CSF viral population suggests that the escape of our patient was due to ongoing viral replication in CNS regions where drug levels were low or her regimen had poor activity. Moreover, this case points out that more than one factor is likely involved with the development of neurosymptomatic CVE and that disproportionate immune response may be associated with acute neurological signs and it may slowly decline after viral control. It also highlights the current lack of certainty about the pathophysiology of this uncommon but serious clinical entity, underlining the need for better understanding CNS viral compartmentalization and the inhibitory activity of antiretrovirals in all CNS cell types.

Conflict of Interest: Giovanni Di Perri has received honoraria from Abbvie, BMS, Gilead, Janssen-Cilag, MSD, Viiv. Andrea Calcagno has received honoraria from Abbvie, BMS, Gilead, Janssen-Cilag, MSD, Viiv and he is currently receiving research grants from BMS, Gilead and Viiv. The other authors declare that they have no conflict of interest.

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Fig 1 Brain magnetic resonance imaging on long-TR sequences at the time of escape (left panel), three (middle panel) and nine (right panel) months later

Extensive signal abnormalities in the form of patchy asymmetrical feeble hyperintensities on long-TR sequences involving both caudate nuclei and thalami, left pallidum, diffusely the subcortical white matter of both temporal lobes, cerebellar peduncles and midbrain until pontobulbar junction were observed at the onset of CVE (left panel). After gadolinium administration, the lesions were non-enhancing. The extension and intensity of these abnormalities decreased over time after HAART modification: basal ganglia presented normal signal on all sequences at 3 months (middle panel), while after 9 months only mesial and polar temporal lobes hyperintensities mildly persisted bilaterally (right panel).

Fig 2 Deep sequencing analysis of viral RNA isolated from the cerebrospinal fluid of our patient at the onset of neurological symptoms

The V1-V3 region of the HIV *env* was sequenced using the Primer ID method (Zhou et al. 2015).

The diverse nature of this population indicates that it is being produced by a large number of HIV infected cells within the CNS.