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News and Commentary

# Cell death mechanisms in HIV-associated dementia: the involvement of syncytia

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HIV-1 affects the central nervous systems in two ways: directly, producing distinct neurological disease, or indirectly, by causing immunodeficiency with increased susceptibility to opportunistic infections and neoplasms. The typical presentation of HIV-associated dementia (HAD) is characterized by cognitive, behavioural and motor dysfunction, and has been defined as a subcortical dementia.[1] Direct neurological involvement includes motor disturbances, cognitive impairments and behavioural changes in various degrees of severity.[2] The initial symptoms of HAD can be subtle and overlooked, or misdiagnosed as depression, and mild forms are known as HIV-1-associated minor cognitive/motor disorders (MCMD) and are characterized by impaired motor speed, working memory and attention, whereas episodic memory and visuo-constructive abilities are relatively unaffected. In the early stages of HAD, memory loss, mental slowing, reading and comprehension difficulties and apathy are frequent complaints. More advanced forms of HAD are characterized by psychomotor slowing, severe memory impairment, disturbances in complex attentional tasks and executive functioning, as well as behavioural manifestations. New onset psychiatric disorders, or a heightened sensitivity to neuroleptic agents, can also be observed. All these findings define a well-characterised syndrome observed during the historical years before the introduction of antiretrovirals. More recently, a severe form of leukoencephalopathy was also reported in HIV-1-infected individuals.[3] This syndrome developed in patients failing highly active antiretroviral therapy (HAART), and the neuropathological features included widespread myelin loss, axonal injury, microgliosis, astrogliosis, and intense perivascular infiltration by monocytes/macrophages and lymphocytes.

HAD commonly occurred during the late stage of HIV-1 infection, when the CD4+ T lymphocytes were most severely depleted, and, even though it occasionally may develop before profound immunosuppression, in general, it results as rare among healthy HIV-1-infected persons. According to historical data, prevalence of HAD was only 0.4% during the asymptomatic phase of infection, but increase to 16% among patients with symptomatic HIV-1 infection. Before the advent of highly antiretroviral therapy, the cumulative risk of developing HAD was estimated to be 15–20%.[4] The HAART introduction resulted in a marked reduction in incidence and severity of opportunistic infections and improvements in survival of patients with HIV-1 infection as well as with a decreased incidence of HIV dementia.[5,6]

Concerning the role of specific determinants of antiretroviral activity over neuroprotection, a strict relationship of cerebrospinal fluid (CSF) viral replication and development, progression and severity of HAD has been well assessed.[7,8] Although, recently a significantly greater neurocognitive improvement was observed among those who achieved virological suppression in CSF. Improvements in cognitive performance were greater among subjects attaining complete suppression of CSF viral load than among those in whom CSF virus remained detectable despite HAART.[9] Even though the effective role of CSF drug penetration over HIV-related neurologic damage still remains a controversial argument,[9,10] the evidence of poor CSF penetration of several antiretroviral drugs[11] could suggest a progressive risk of ineffective control of CSF HIV replication in long-term-treated patients. The inefficient control of CSF viral replication, in addition to the impaired neurocognitive function, constitutes a potential virus reservoir for new systemic infections. In fact, the observation concerning the onset of new forms of acute HIV encephalitis in chronically infected individuals, putatively due to the central nervous system (CNS) escape of HIV-1 replication, indirectly confirmed the use of CNS-penetrating HAART in patients with neurological complaints.[12] These findings actually suggest that inhibiting HIV-1 replication in the CNS may reduce viral neuropathogenicity, allowing neuronal repair and restoration of cognitive function, and supporting the evidence that CNS damage in HIV-1-infected persons could be more directly related to HIV replication in CNS tissues than it is systemically.[9]

## Mechanism(s) of HIV-related neuronal damage

The pathogenetic mechanism(s) that is the basis of HIV-1-induced neuronal injury as well as the role of the virus has not been fully elucidated yet. HIV-1 enters the CNS early in the course of infection through infected macrophages and resides both in macrophages and microglia, while neurons are not productively infected by HIV.[13,14] Several studies have proposed different ways by which HIV-1 may lead to neuropathology. One theory hypothesises that the virus induces neuronal injury by the release of neurotoxic viral

proteins that can be directly deleterious for neurons. Many evidences support this theory; in fact, the viral proteins Tat, Nef, Vpr, Rev, gp 120 and gp 41 have been shown to cause neuronal injury.[14] Recent studies emphasise the importance of the envelope virus proteins in inducing neuronal toxicity.[15] gp 120 causes neuronal dysfunction and death *in vivo* in rodents.[16,17] The viral proteins can interact with several receptors on neurons; presumably, the viral proteins interact with chemokine receptors since the interruption of chemokine receptor signalling can prevent HIV-induced neuronal apoptosis.[18]

*In vitro* experiments demonstrated that the ability of the virus to induce neuronal apoptosis is independent from the replication capacity. In fact, the presence of the viral envelope protein gp120 at the cell membrane is sufficient for the induction of neurotoxic mechanisms despite the lack of replicative capability of some virus strains.[15,19] However, it is important to emphasise that the majority of these data are obtained by *in vitro* models that could not exactly reproduce the *in vivo* setting. In addition, although some viral proteins have been detected in body fluids such as gp120, it is not clear whether their concentration is sufficient to induce cell death *in vivo*.[20]

A second theory describes an indirect role of the virus. The description that apoptotic neurons normally do not colocalise with infected microglia in HIV patients is the best evidence supporting the indirect involvement of the virus in generating neuronal apoptosis.

Macrophages and microglia can be activated by HIV, and also by factors released from infected cells such as cytokines and/or shed viral proteins such as gp120.[13,14,21] Some of these molecules can be neurotoxic such as quinolic acid, glutamate, nitric oxide and other reactive oxygen species, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), platelet-activating factor (PAF), and extracellular matrix degrading enzymes (MMPs).[14,22–24] Among cytokines, interleukin-1$\beta$ (IL-1$\beta$) and TNF-$\alpha$ have been shown to be overexpressed in the nervous system of HAD-affected patients.[13,14] The chemokines and their receptors present on macrophages, microglia, neurons and astrocytes carry out an important role in HIV-induced damage.[14,25] In fact, HIV-1 enters into macrophages/microglia or T cells by the binding of the envelope viral protein gp120 to chemokine receptors in conjunction with CD4. The chemokine receptors are subdivided into four families: $\alpha$-chemokines, $\beta$-chemokines, fractolkine and lymphotactin. To date, at least 10 chemokine receptors have been shown to act as coreceptors for HIV-1;[26] some of these receptors mediate HIV-1-associated neuronal damage whereas others may play a protective function. In fact, the stimulation of $\alpha$-chemokine receptor CXCR4 can induce death pathway(s); by contrast, the activation of $\beta$-chemokine receptors CCR5 seems to play a neuroprotective role.[25,26] The viral proteins or the neurotoxins can, alternatively, interact with the *N*-methyl-D-aspartate receptor (NMDAR), thus suggesting another way directly eliciting neuronal death.[14]

## Cell death pathways in HAD

The biochemical pathways of HIV-induced neurodegeneration are not well elucidated. Several experimental evidences indicate that the neuronal loss is essentially due to apoptosis induction.[27] More recently, utilising animal models, it has been shown that HIV gp120 is neurotoxic *in vivo* and activates a caspase-dependent apoptotic pathway.[28] Apoptosis induction in neurons can be mediated by different stimuli. One possible way is the overstimulation of NMDA receptor triggered by neurotoxins released from the infected or immune-stimulated macrophages/microglia.[14,25] The stimulation of NMDAR determines an excessive influx of $Ca^{2+}$ into neurons that triggers the activation of p38 mitogen-activated protein kinase (MAPK) and an overload of $Ca^{2+}$ in mitochondria, leading to the generation of reactive oxygen species, release of cytochrome *c*, caspase activation and apoptosis.[14,17] In keeping with the role played by NMDAR in HIV-associated dementia, the inhibition of NMDAR pathway by its antagonist memantine prevents the neuropathological alterations in a mouse model of HAD.[29] Alternatively, HIV-1 proteins can stimulate the chemokine receptors such as the $\alpha$-chemokine receptor CXCR4 that induces apoptosis. In contrast, the stimulation of $\beta$-chemokine receptor CCR5 shows neuroprotective effects by uncharacterised pathway(s).[14,25] In addition, infected or activated microglia can release inflammatory cytokines such as TNF-$\alpha$ or IL-1$\beta$ that themselves induce apoptosis. In keeping with this hypothesis, the TNF-$\alpha$ and its receptor levels are elevated in HAD patients.[30] One way by which TNF-$\alpha$ is capable of stimulating apoptosis in neurons is mediated by the TNF-related apoptosis-inducing ligand (TRAIL). TRAIL is one member of the TNF superfamily, and the TRAIL receptors are transmembrane proteins with a cytoplasmic death domain that can induce apoptosis.[31] The HIV infection upregulates TRAIL and TRAIL-overexpressing macrophages were observed in human brain tissue obtained from a patient with HAD.[32] Interestingly, these TRAIL-positive macrophages are found in association with caspase-3-positive neurons, suggesting a possible role of TRAIL in mediating neurotoxicity.[33]

Recently, the involvement of p38 MAPK in neuronal apoptosis has been demonstrated; its activation results in the release of cytocrome *c* from mitochondria, caspase activation and chromatin condensation.[14,17] In fact, p38 MAPK inhibitors abrogate neuronal apoptosis due to gp 120 exposure.[14,34]

p53 has been proposed to be a major player in mediating the cell death pathway during HAD.[34] p53 is a proapoptotic transcription factor that activates the expression of genes involved in growth arrest or cell death in response to multiple forms of cellular stress.[35] In addition to neurons, p53 overexpression has been described in glial cells present in the brain of HAD patients.[34] *In vitro* experiments revealed that p53-dependent signalling is required for apoptosis induction both in neurons and microglia. Taken together, these findings suggest that p53 is an essential proapoptotic regulatory factor in HAD.[34] Recently, we demonstrated the involvement of a p53-dependent apoptotic pathway in HIV-1-induced syncytial apoptosis (see Perfettini *et al.*, the same CDD issue). p38 MAPK has been shown to be the main kinase activating p53 through its phosphorylation on ser 46. The overexpression of these proteins has been detected in the syncytia present in both the lymph nodes and the brains of HIV-1-infected patients.[36] p38 MAPK involvement in macrophages/microglia cell death during HAD has been well documented. In fact, the

direct infection or the stimulation of chemokine receptors CXCR4 or CCR5 in conjunction with CD4 triggers a signal pathway that involves p38 MAPK.[14] In keeping with these findings, for the first time, we recently described the presence of p38 MAPK and also of p53S46 in HIV-1-induced brain syncytia in patients with HAD,[36] thus suggesting their participation in macrophages/microglia cell death (Figure 1). p53 is activated and mediates cell death also in PBMCs and lymph nodes from HIV-infected patients; the activation of p53 is mediated by the factor mTOR/FRAP.[37] mTOR/FRAP is the mammalian target of the rapamicyn, which phosphorylates p53 on ser 15, leading to its activation with the consequent induction of the mitochondrial death pathway[37,38] by controlling the expression of the proapoptotic components of Bcl-2 family such as Puma and Bax. Puma binds Bcl-2 and localises on the mitochondria where it induces cytochrome *c* release and the consequent activation of caspases.[37–40] Caspase-3 activity has been detected in brain patients with HAD[17] and *in vitro* infected neurons and macrophages.[18] In addition to the important role played by caspase-3, the activation of caspase-8 and caspase-9 in neurons upon stimulation by cytokines or excitatory amino acids has been demonstrated.[17,41] The inhibition of caspase-8 and caspase-9 prevents the activation of caspase-3 and neuronal apoptosis, thus suggesting that they act upstream of the apoptosome.[41] Moreover, a recent analysis performed on apoptotic neurons from HAD patients revealed a significant increase of caspase-2 mRNA.[42] In keeping with the role played by caspases is the finding showing that their inhibition prevents the neuronal degeneration of CNS observed in HIV/gp120 transgenic mice.[17] We have recently demonstrated that the neuropathology responsible for HAD is often characterised by the formation of multinucleated giant cells or syncytia, which arise from the fusion of non-neuronal cells. We observed the presence of syncytia in the frontal cortex of approximately 50% of HAE patients; the event correlates with a high number of cells expressing the HIV-1 protein p24 or exhibiting apoptotic DNA fragmentation detectable with the TUNEL technique. Histochemical and immunohistochemical analyses revealed that syncytia detected in HIV-infected brain are undergoing apoptosis through a mitochondrial pathway previously delineated for HIV-1 envelope-elicited syncytia *in vitro*. In fact, we observed the overexpression of the mammalian target of mTOR and the consequent overexpression of two potential effectors of mitochondrial apoptosis, namely the 'BH3-only' proteins Puma and 'tissue' transglutaminase (TG2). HAD syncytia also manifested the phosphorylation of IκB, which is a sign of the activation of NF-κB, a transcription factor that may cooperate with p53 in the induction of syncytial apoptosis (Figure 1). Altogether, these findings provide clues on the mechanisms of cell loss occurring in HIV-associated encephalitis.

In conclusion, the recent evidences show that HAART, although attenuating the severity of neurological disease, does not definitively eliminate HIV-1 infection in the CNS and the recent rising prevalence detected in the last years in infected patients highlights the need for alternative treatment for this HIV-dependent pathology.[9–11] Particularly interesting is the finding demonstrating the formation of dying syncytia,

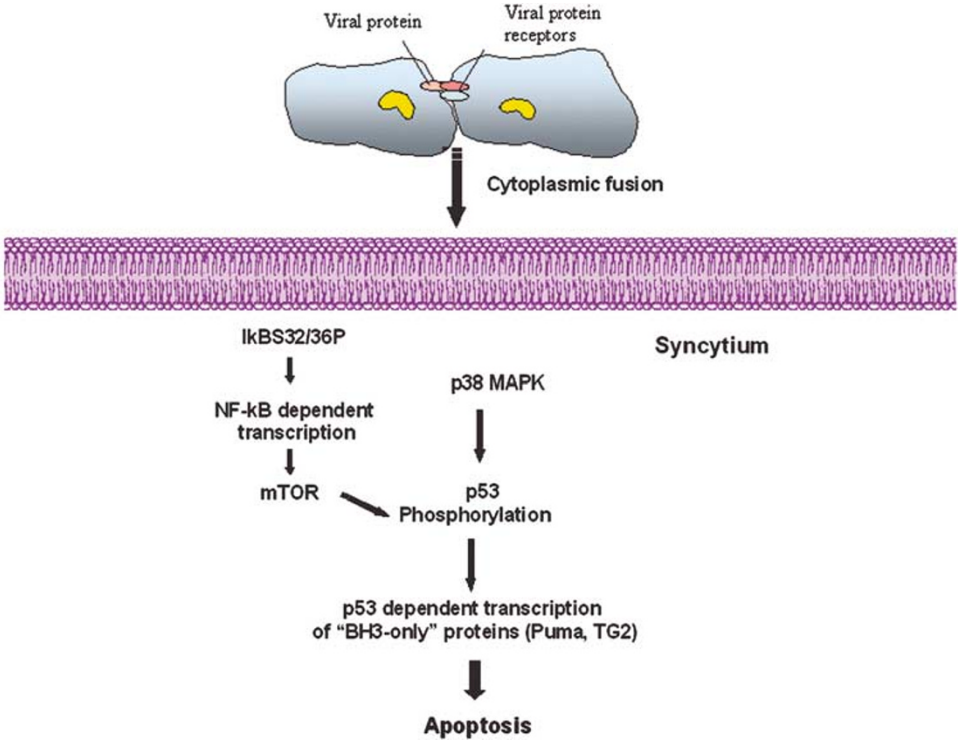


**Figure 1** Hypothetical mechanisms of HIV-1-mediated macrophages/microglia killing in HAD pathogenesis. HIV-1-infected macrophages/microglia can fuse with uninfected cells expressing a suitable combination of receptors and generate a syncytium designed to death. Syncytia manifested the phosphorylation of IκB that is a sign of the activation of NF-κB. mTOR and p38 MAPK are two kinases, activated by cell fusion, that phosphorylate p53. p53 phosphorylation induces the overexpression of two potential effectors of mitochondrial apoptosis, namely Puma and TG2

which may play an important role not only as a virus reservoir but also as a pathogenetic event in HAD. In keeping with this, future researches should fully characterise the cell death pathways involved in HAD, with the purpose of defining new molecules that can block apoptosis in the brain.

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