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***In vitro* models of HIV-1 infection of the Central Nervous System**

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Abstract

Neurocognitive disorders associated with HIV-1 infection affect more than half of persons living with HIV (PLWH) under retroviral therapy. Understanding the molecular mechanisms and the complex cellular network communication underlying neurological dysfunction is critical for the development of an effective therapy. As with other neurological disorders, challenges to studying HIV infection of the brain include limited access to clinical samples and proper reproducibility of the complexity of brain networks in cellular and animal models. This review focuses on cellular models used to investigate various aspects of neurological dysfunction associated with HIV infection.

Introduction

Before the introduction of the antiretroviral therapy (ART), the brain of HIV/AIDS patients presented with extended areas of inflammation due to viral replication and microglia activation, causing encephalopathy [1–3]. At present, however, with an effective ART treatment that keeps the virus at very low or undetectable levels, HIV patients rarely develop encephalopathy. Still, over 50% of those infected will develop neurological problems. The HIV-associated neuropathology (also called neuroHIV) includes alterations in neuronal and glia function, both of which are exacerbated by the abuse of alcohol and drugs. *In vitro* cellular models provide important tools to assess molecular mechanisms underlying neuronal damage or immune and glia activation. Since its first description as AIDS dementia in the middle 80's [4–9], thousands of published studies have allowed to acquire valuable information on the mechanisms of neuronal dysfunction associated with HIV infection using cellular and animal models. Here, we review cellular models in neuroHIV (summarized in Table 1), as animal models have been reviewed in great details elsewhere [10–14]. As there are numerous studies investigating neurotoxicity due to HIV infection, we apologize for not citing all the literature.

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Conflict of interests statement

The Authors declare no conflict of interests.

Cell lines and primary cell models

Primary rodent and human cells and cell lines are used to investigate various aspects of viral protein-mediated cellular toxicity, as well as studying the interaction between viral proteins and opioids, common comorbidity factors in persons living with HIV (PLWH). The effect of viral proteins and cocaine, alone or in combination is investigated on isolated human or rodent primary neurons, astrocytes, or in mixed cultures of neurons and glia. The general flow for neuroHIV research begins with an initial observation in clinical samples acquired from HIV-positive patients (typically archived brain tissue, cerebrospinal fluid or brain imaging), followed by *in vitro* experiments using primary cells, cell lines, induced Pluripotent Stem Cells (iPSCs), organotypic brain slices, or mixed cultures derived from the combination of any of those cellular systems; results are then further validated using animal models or HIV clinical samples (i.e. immunohistochemistry, *in situ* hybridization, etc...). These type of studies have allowed for the characterization of cellular events involved in neuronal toxicity, including toxicity due to viral proteins such as Tat, gp120 and Nef. Perhaps one of the first mechanisms of neurotoxicity demonstrated using primary neuronal cultures was calcium dysregulation [15–24] and apoptotic cell death induced by HIV-1 gp120 [25–29] and Tat [17,30–37]. Oxidative stress has also been extensively investigated using *in vitro* cellular models [15–24,36,38–46]. Primary rat astrocytes were used to determine the neurotoxic effects of three antiretroviral compounds, maraviroc, darunavir and raltegravir, alone or in combination after simultaneous exposure to lipopolysaccharide (LPS) [47]. All three drugs induced the production of reactive oxygen species (ROS) and the inhibition of matrix metalloproteinase 2 (MMP2) and MMP9 [47]. Additionally, peroxisome proliferator-activated receptor γ (PPAR γ) agonist rosiglitazone reversed the gp120-mediated inflammatory response of primary mixed cultures of rat astrocytes and microglia [48].

Cellular models are utilized to investigate inflammatory pathways involved in neuroHIV. Infiltration of monocytes/macrophages into the brain parenchyma is critical to HIV-mediated neuroinflammation and neuropathogenesis [49–51], even in the combination ART (cART) era [52,53]. Zheng et al. utilized human fetal primary astrocytes to show that HIV-infected or immune-activated macrophages secrete tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β), which in turn stimulate astrocytic production of chemokine C-X-C motif ligand 8 (CXCL8) [54]. Secreted IL-1 β can also result in production and secretion of stromal cell-derived factor 1 (SDF-1) by astrocytes, contributing to neuropathogenesis [55]. Human fetal primary astrocytes were used to investigate inflammation and the effect of the anti-retroviral drug abacavir in the induction of endoplasmic reticulum (ER) stress in those cells [56]. Regarding inflammatory mechanisms, the inflammasome component nucleotide-binding oligomerization leucine rich repeat and pyrin domain containing 3 (NLRP3) has been found to be activated by HIV-1 infection in macrophages [57], and polymorphisms of this gene have been linked to increased susceptibility to HIV-1 infection [58,59]. Rapid increase of inflammasome proteins in microglia has been found to contribute to HIV-associated brain disease [60]. Among the mechanisms implicated in the activation of the inflammasome in microglia, the viral proteins Tat and R have been found to contribute to neuroinflammation [61–63]. The leucine-rich repeat kinase 2 (LRRK2) is an important regulator of microglia activation during inflammation [64], and its inhibition attenuates Tat-induced pro-

inflammatory responses *in vitro* and *in vivo* [65]. Recently, using cultured human astrocytes, Ojeda et al. showed selective mitochondrial degradation (mitophagy) as a mean to counteract inflammation when the infection is productive, while astrocytes exposed to the virus in an abortive module showed mitochondrial damage, inflammasome activation and cell death [66]. The importance of mitophagy in the development of HIV-associated neurological disorders was additionally established in human neurons [67]. Exposure of neurons to gp120 and Tat accelerates mitochondria fragmentation through mitochondrial dynamin-related protein 1 (DRP1), increased microtubule-associated protein light chain 3 beta (LC3B) and selective recruitment of Parkin/SQSTM1 (sequestosome 1) to the damaged mitochondria [67]. In mouse primary neuronal cultures, HIV-vpr protein was additionally shown to disrupt mitochondria axonal transport and accelerate neuronal aging [68].

The effect of viral proteins on the cytoskeleton and on synapto-dendritic function and integrity has been observed in the brain of HIV-infected individuals with encephalitis [69]. Few years later, several details in the molecular events underlying neurite function have been identified. For instance, elevated levels of osteopontin observed in the plasma, brain and CSF of HIV-positive individuals [70,71] blocks the negative effect of HIV env on neurite growth via an integrin/TORC1/2 signaling pathway [72]. Rat hippocampal neurons were utilized to determine the actin cytoskeleton-mediated attenuation of an N-methyl-D-aspartate receptor (NMDAR)-evoked increase in intracellular calcium in response to HIV-Tat [73]. Microtubule associated protein 2 (MAP-2) mediated intracellular trafficking from the endoplasmic reticulum (ER) to the Golgi apparatus (GA) of the complex mannose binding lectin (MBL)/gp120 [74]. Related to the protein MAP-2, our lab has shown that Tat causes rapid degradation of this structural protein and degeneration of cytoskeletal filaments. The mechanism involves Tat-mediated translocation of the proteasome to the site of microtubule filaments [75]. We additionally found that Tat promotes aberrant splicing of Tau exon 10 through increased levels of the serine/arginine rich splicing factor SC35, which is retained in nuclear speckles [76]. The mechanism further involved Tat-mediated increased expression of the dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) [76]. A proteomic approach was used to model HIV-infected microglia and astrocytes crosstalk. In this study, the interaction of vesicular stomatitis virus pseudotyped HIV infected murine microglia with astrocytes showed extensive cytoskeletal rearrangements that modulated cell death and migratory pathways [77].

Among the cell lines, human neuroblastoma SK-N-SH, and its derivative SH-SY5Y, mouse neuro2A and rat PC12 can be differentiated into various neuronal subtypes through their stimulation with different compounds. Amid the most recent findings, Gerena et al. have shown the presence of soluble Insulin Receptor (sIR) in the cerebrospinal fluid of HIV⁺ patients with neurocognitive dysfunction, and that the viral protein Tat can trigger the release of sIR from cultured SH-SY5Y neurons [78]. SH-SY5Y and mouse primary neuronal cells were also used to show dysregulation of the transcription factor E2F3 by Tat [79]. The rat B103 neuroblastoma cultures and *in vivo* models were used to show that the anticancer drug sunitinib protects from Tat-induced neurotoxicity *via* activation of autophagy [40].

Using human fetal brain cultures Nath et al. demonstrated synergistic neurotoxicity between gp120 and Tat, which can be prevented by memantine [80]. Primary rat fetal neurons were used to demonstrate the rapid induction of endoplasmic reticulum (ER) stress by Tat [81].

The most abundant brain cells involved in neuronal plasticity and protection are astrocytes, which have been extensively investigated as possible HIV reservoirs, given that viral particles, DNA, RNA, and proteins were detected in these cells, even though the mechanism of viral entry were unclear unknown [82–91]. Failure to detect CD4 receptor in astrocytes parallels with lack of viral replication in these cells [92], a concept that has led to the hypothesis that astrocytes can uptake and release viral particles by endocytosis [83,93–95]. However, more recently, using advanced technologies Russell R et al. elegantly demonstrated that astrocytes are not infected by HIV, but that they phagocytose viral material from infected macrophages [96]. Recent literature using highly sensitive *in situ* hybridization techniques, RNAscope and DNAscope demonstrated the presence of viral RNA, but not DNA, in astrocytes in brains of virally suppressed individuals [97].

Organotypic explant models

In addition to dissociated neuronal cell cultures, organotypic brain slices offer a tridimensional cellular system that allows a more complex simulation of *in vivo* conditions [98]. While this *in vitro* model is still employed to investigate mechanisms of neuronal damage in neurodegenerative disorders, it has been not fully explored in the field of HIV. In fact, only 13 articles contain the keywords “organotypic brain slice” and “HIV” up to year 2011. Nevertheless, the first study using rat hippocampal slices explants was in 1996 and demonstrated neurotoxicity mediated by HIV-1 envelope protein gp120 [99]. Co-culture of the hippocampal explants with macrophages provided a more complex model of HIV-induced neurotoxicity and brain damage [100]. Treatment of mouse organotypic hippocampal slice cultures with Tat resulted in increased production of indoleamine 2, 3 dioxygenase (IDO) in an interferon γ (IFN γ)-independent but p38 mitogen-activated protein kinase-dependent manner [101]. Rat organotypic hippocampal slices were used to study the effect of recombinant Tat on microtubule formation and oxidative stress-related neuronal injury [38]. Human fetal organotypic brain slices placed on semipermeable hydrophilic membrane inserts on top of wells containing cultured HIV-infected T cells, allowed for the study of the effect of HIV, HIV proteins, and other molecules released by the infected T cells on neuronal cells [102]. The authors suggest this model could be used to study the dynamics and the microenvironment of brain tissue exposed to HIV-1 [102]. In another study, pre-treatment of a rat hippocampal-entorhinal slice with moderate amounts of ethanol protects against pro-inflammatory HIV-1 gp120 protein [103]. Long term ethanol exposure, however, sensitizes the hippocampus to the neurotoxicity mediated by HIV-1 Tat in a NMDA receptor-dependent manner [104].

Neuronal progenitor models

Models of HIV-associated neuronal damage include human and rodent progenitor cells and iPSCs. These models have been successfully explored for neurodegenerative disorders, including Alzheimer's, Huntington's and fronto-temporal dementia [reviewed in [105]]. Rat

E15 brain neuronal progenitors were used to investigate neurons and astrocytes response to signaling by SDF-1 and gp120 [106]. Our laboratory has also utilized rat neural progenitors to determine calpain-mediated cleavage of Tat and the role of cleaved Tat isoforms in neurotoxicity [107]. Mouse neural progenitor cells (NPCs) served as a model to investigate the neurotoxic effect of combined antiretroviral therapy [108]. The authors found that a combination of three drugs, tenofovir disoproxil fumarate, raltegravir and emtricitabine, inhibits proliferation and induces apoptosis of progenitor cells [108]. Methamphetamine treatment increased HIV production and decreased neuronal differentiation in mouse and human neural progenitors [109]. On the other hand, platelet derived growth factor BB (PDGF-BB) signaling pathways seem to protect the negative action of cocaine and HIV-Tat on neural progenitor differentiation [110]. Using human NPCs, it was observed that expression of Tat in primary astrocytes resulted in deregulation of miRNAs, particularly miR-320a, and enhanced ATP release. The mechanism involved voltage-dependent anion channel protein 1 (VDAC1) upregulation upon the Tat-induced downregulation of miR-320a [111].

Blood brain barrier (BBB) models

The protective effects of the blood brain barrier (BBB) on the brain is compromised by HIV infection [recently reviewed in [112,113]]. In particular, the density of pericytes is reduced in HIV-associated neurocognitive disorder [114,115], and their function is compromised allowing monocyte infiltration and overall leakage [115]. Commercially available primary human brain vascular pericytes were used to demonstrate that these cells can efficiently silence HIV-1 expression, and that viral latency renders pericytes sensitive to the excess of extracellular glutamate [116]. Oda et al. established a bilayer model of BBB using astrocytes and endothelial cells on 3µm polyethylene terephthalate (PET) membrane inserts, which were cultured in growth medium for seven days before exposure to HIV⁻ or HIV⁺ peripheral blood mononuclear cells (PBMCs) [117]. As suggested by the authors, this model could be useful to assess the HIV DNA copy numbers of transmigrated cells pre- and post-targeted ART and to understand the role of oxidative stress related to HIV DNA and HIV-associated neurocognitive impairment [117]. Even if HIV infects only about 5% of pericytes [118], the propagation of injury signals can occur to neighboring cells through gap junctions [119]. Human immortalized cerebral microvascular endothelial cell line (hCMEC/D3) was used to test polymeric nanoparticles for HIV therapy [120], and to investigate the effects of HIV-1 Tat and methamphetamine on BBB integrity and function [121]. The use of biodegradable nanoparticles crossing the BBB has been explored to improve brain delivery efficiency of antiretroviral drugs and is reviewed elsewhere [122]. Human brain microvascular endothelial cells (HBMEC) obtained from adult brain tissue are often utilized to model BBB *in vitro*. These cells have been used to show increased expression of HIV-induced pro-inflammatory factors [123,124]. Bhargavan et al used this model to investigate molecular details of endothelial inflammation and BBB dysfunction caused by HIV-induced Toll-like 3 receptor (TLR3) activation [125]. Exposure of HBMEC to HIV resulted in increased release of extracellular vesicles (ECV) carrying amyloid β from these cells [126]. Interestingly, exosomes shed by HBMECs are able to transport antiviral molecules to macrophages, as a possible defense mechanism against HIV invasion of the CNS [127]. Human vein

endothelial cells (HUVECs) treated with antiretroviral drugs underwent premature senescence associated with inflammation and oxidative stress, which had detrimental effects on astrocytes [128]. The same cellular model has been used to investigate the effects of viral proteins on the BBB [129], or the transendothelial migration of CD16⁺ monocytes under pro-inflammatory stimuli [130].

Conclusions

Finding the best cellular model to recapitulate all aspects of a disease is critical for the advancement of our knowledge in the field and to devise proper therapy. Neurological disorders have historically challenged our ability to reproduce the complexity of the brain *in vitro*, and HIV-1 infection is no exception. The infection itself evolved with the introduction of combined antiretroviral therapy, presenting with less obvious signs of inflammation due to viral replication, as well as less cell death, though neuronal cells show an increase in subtle damages overall. Even with all the undisputable limitations, cellular models remain an invaluable tool to investigate biological and molecular aspects of HIV infection of the CNS, as they allow us to advance the field.

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Table 1.

Summary of cellular models discussed in this review.

Cellular model	Description	References
Cell lines	SK-N-SH, SH-S5Y5 and B103, neuro2A, PC12	40, 41, 42–48, 79
Primary cells	Astrocytes, neurons, mixed cultures glia/neurons, iPSCs	15–37, 39, 54, 55, 56, 66, 67, 68, 73, 74, 76, 77, 80, 81
Organotypic explants	Human fetal or rodent brain slices	38, 99–104
Neuronal progenitors	Primary human or rodent neural progenitors or commercially available iPSCs	105–111
Blood brain barrier models	Primary human or rodent pericytes, bilayer with astrocytes and endothelial cells, human brain microvascular endothelial cells, human immortalized endothelial cell line (hCMEC/D3), human vein endothelial cells (HUVEC)	116–130