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Journal of AIDS and HIV Treatment Commentary

Brain Organoids: An Emerging Model System to Study HIV-1 Neuropathogenesis

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Received date: July 07, 2021, **Accepted date:** October 07, 2021

Chronic Human Immunodeficiency Virus (HIV-1) infection in the brain results in mild cognitive, motor and sensory deficits in more than 50% of people living with HIV-1 (PLWH), despite systemic viral suppression [1]. These symptoms are collectively termed as HIV-associated neurocognitive disorders (HAND). HIV-1 enters the brain during the acute phase of infection establishing an inflammatory microenvironment that provokes changes in neuronal structure and function, particularly in the frontal cortex [2,3]. These structural and functional changes in neurons correlate with deficits in memory and cognition, increasing the risk of poorer health outcomes in PLWH under antiretroviral treatment [4]. Currently, there is no treatment that can prevent or restore the neuronal damage and cognition, suggesting that there is still much to learn about the underlying mechanisms of HIV-1 infection in the brain.

Because of the lack or limited availability of post-mortem human donor brain tissue samples representing the different stages of HAND, the studies of viral-host interaction during the onset of HAND relied mainly on *in vitro* neuronal cell cultures or small animal models that may not accurately mimic the HIV-1 infection of human brain. Hence, a system which recapitulates the cellular and molecular complexity of the human brain is urgently needed to further the studies on neuropathogenesis and to develop therapeutics. More recently, notable advancements have been made in the development of brain organoids [5]. Brain organoids are three-dimensional culture structures generated from either induced pluripotent stem-cells (iPSC) or neuroprogenitor cells (NPC) which under specific conditions mature and differentiate into brain cell lineages and organize functionally corresponding to human brain *in vivo* [6-8]. Indeed, brain organoids have become increasingly popular for providing a valuable platform to study neurogenerative and neuropathological diseases in *in vitro* [9-13]. These brain organoids offer unprecedented advantages for studying human-specific neuroinvasive pathogens representing a more physiologically relevant model of the human brain by overcoming interspecies discrepancies often observed in animal models [14,15]. However, current organoid protocols still have many challenges, including the lack of immune cells such as microglia/macrophages that are particularly important for neuroinflammatory diseases including HIV-1 associated neuropathogenesis [16,17].

We have reported a method to develop 3D-organoid culture system to model HIV-1 neuropathogenesis [11]. Our protocol efficiently generated different neuronal subtypes and astrocytes. To accurately represent HIV-brain infection dynamics *in vitro*, we also introduced microglia, which successfully embedded into the brain organoids to further analyze the pathological processes. These microglia-containing human brain organoids (MG-hBORGs) support low level of HIV-1 replication, similar to infection observed HIV-1 infected human brain [2]. Another key observation in this model is, upon incorporation of HIV-infected microglia, a significant neuroinflammatory response was mounted resulting in astrogliosis and neurodegenerative pathologies similar to the HIV-1 infected individuals' brain [3,2]. Additionally, we also demonstrated increased degeneration and loss of synaptic processes in neurons within the organoids containing HIV-1 infected microglia compared to the organoids containing uninfected microglia [11]. Hence, the MG-hBORG model has the potential to assist investigators to study the molecular mechanism(s) underlying HIV-1 neuropathology.

The primary focus of developing our MG-hBORG model is to study HIV-1 neuropathogenesis using a physiologically relevant model. Ideally, the incorporation of primary immortalized microglia into organoids would recapitulate part of the features of adult human brain. However, the source of adult postmortem brain-derived microglia is still a limiting factor in our model. Stem-cell derived microglia have been shown to be a surrogate for primary adult human microglia, although techniques are complex and protocols are time-consuming [18-20]. Thus, we sought to evaluate the incorporation of the immortalized microglia cell line (HMC3) and immortalized primary adult brain microglia into our organoid model. We noticed an enhanced inflammatory response in HIV-1 infected primary microglia compared to HIV-1 infected microglia, yet both microglial models were readily infected by neurotropic virus and were found embedded in the hBORGs as early as 3 days after incorporation. As an immortalized cell line, HMC3 microglia continued to proliferate at high rates on top of the organoids. Indeed, we observed that HMC3 microglia outnumber the organoids cell number as early as 10 days, limiting the studies to early aspects of HIV-1 neuropathogenesis (data not published). However, incorporation of immortalized primary adult brain microglia resulted in long term organoid culture, due to their slow replication rate.

Although our proposed brain organoids represent an advanced culture system to study HIV-1 neuropathogenesis, further improvements are still necessary to have complete physiological relevance, such as neuronal immaturity, lack of vascularization and blood brain barrier (BBB) [21-23]. Transcriptome studies show that cells constituting the brain organoids present gene expression patterns remarkably close to that observed in fetal brain tissue, raising the question regarding the maturity stage of these organoids and their relevance as models for late and aging related neurodegenerative disorders in adults [24]. Therefore, an optimal differentiation and maturation protocols that mimics *in vivo*-like adult brain cell composition and functional characteristics are needed.

The susceptibility of human macrophages/monocytes to HIV-1 infection is well documented in both *in vitro* and *in vivo* [25-27]. For instance, circulating monocytes are permissive to HIV-1 infection and infiltrate the brain differentiating into infected resident macrophages [28,29,1]. These infected macrophages serve as early amplifiers of the virus releasing new HIV-1 particles, viral proteins, cytokines and chemokines, which in turn may activate nearby uninfected macrophages and potentially glial cells [30]. Thus, the lack of other immune cells in brain organoids as macrophages represents an important limitation of the current organoid models to study neuroinflammatory diseases. Therefore, the presence of macrophages along with microglia will strengthen the physiological relevance of viral spread and immune activation induced by HIV-1 in the brain.

A further challenge in the use of brain organoids to study HIV-1 neuropathogenesis is the lack of vascularization. The reduced infiltration of nutrients and oxygen into the core of the brain organoids inevitably results in tissue necrosis, reducing their viability for long-term neurodegenerative studies. Furthermore, low fluid penetration into the brain organoids may also affect studies using antiretrovirals and/or screening of new therapeutics due to variations on drug concentrations along the organoid's radius. Thus, the integration of a vascular structure within the brain organoid remains a major challenge and requires further work to optimize such culture conditions.

Although the antiretroviral therapy was of great success to suppress viral replication, there is still no cure for HIV-1 and comorbidities are now the major challenge for people living with HIV-1. Among the comorbidities, HAND represents health, social and economic burdens impacting the overall well-being of the HIV-1 population [4]. Despite the high incidence and prevalence of this disease, the neuropathogenesis underlying the cognitive impairment is still unfolding. In this regard, brain organoids will allow us to further our understanding on host-pathogen interactions and to elucidate mechanisms aimed to identify new potential drug targets for treatment to combat HIV-neuropathogenesis.

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