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A microglia-containing 3D human brain organoid for studying HSV-1-induced Alzheimer's disease

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Abstract:

Alzheimer's disease (AD), recognized for its profound incapacitation, presents with notable cognitive decline and memory deficits. A growing body of research points to a link between infection with herpes simplex virus type 1 (HSV-1) and the exacerbation of AD. Our study pioneers the use of a cutting-edge, three-dimensional human brain organoid model enriched with microglia, referred to as MC-HBO, to explore the mechanisms by which HSV-1 influences the pathogenesis of AD. Through this model, we examined the regulatory effects of cytokines on amyloid-β (Aβ) deposition, a cardinal pathological manifestation of AD. Our findings demonstrate that the suppression of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), leads to a significant reduction in Aβ deposition. In contrast, the inhibition of anti-inflammatory cytokines, such as interleukin-10 (IL-10) and interleukin-4 (IL-4), was correlated with an increase in Aβ accumulation. These insights into the role of cytokine signaling pathways suggest a promising therapeutic strategy for potentially decelerating the progression of AD.

Keywords: Alzheimer's disease (AD), Herpes simplex virus type 1 (HSV-1), Amyloid-β (Aβ) plaques, Tau proteins, Neuroinflammation.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to the gradual worsening of the patient's cognitive function[1]. Some of the symptoms include memory loss, language deficits, and personal identification. In the later stages, patients also exhibit symptoms of personality disturbance and typically die on average 7 years from the first diagnosis[2]. However, the cause of the disease remains complicated. More and more evidence shows that HSV-1 has potential causality in AD.[3]

The hallmarks of AD are the build-up of highly phosphorylated tau protein inside cells and the development of amyloid-β (Aβ) plaques outside of cells, which result from the amyloid precursor protein's proteolytic breakdown. [4]. The degeneration of synapses and neurons is linked to these important protein clusters. The AD brain has neuroinflammatory alterations in addition to protein aggregation.[5] Microglia play a key role in neuroinflammation. [6] When activated by immune responses, microglia adopt a reactive state, altering both their shape and function[7]. Based on the M1/M2 macrophage classification, activated microglia were initially classified as either pro-inflammatory 'M1' or reparative 'M2' phenotypes.

The primary immune cells in the central nervous system (CNS), microglia account for 10–15% of all brain cells. They play a crucial role in maintaining homeostasis within the CNS and respond to pathological changes.

Microglia can exist in different states, notably the M1 and M2 phenotypes. M1 microglia are linked to a pro-inflammatory response, producing cytokines such as TNF-α and reactive oxygen species, which drive NLRP3-mediated neuroinflammation and lead to neuronal cell death. Conversely, M2 microglia are recognized to have a more 'healing' function, being less inflammatory.In the M2 state, microglia are involved in neurogenesis and are pivotal in the removal of aggregated proteins that are detrimental to the neurons. Nevertheless, such animal models as mice do not possess human genetic features and therefore cannot fully mimic the pathological symptoms of AD. Thus, there is a great demand for an in vitro human brain organoid.A way to improve the realism of human brain organoids is to include microglia, a cell type of significant importance in the CNS and AD.

In the present work, we aimed at understanding the involvement of microglia in the course of AD by employing human-induced pluripotent stem cells (hiPSCs) to derive human brain organoids with microglia (MC-HBO).

2. EXPERIMENT

2.1 Generation of Microglia-Containing Human Brain Organoids (MC-HBOs)

The (MC-HBOs starts by differentiating hiPSCs into two types of cells: neural precursors and microglia. Neural precursor cells are introduced into a three-dimensional gel support and develop into HBOs. At the same time, another culture medium is used to induce another group of hiPSCs into microglia. The experiment includes two groups: The experimental group included brain organoids with human microglia, and the control group included brain organoids without microglia.

2.2 Infection with HSV-1:

MC-HBO and control HBO cultures without microglia are both cultured for 33 days.Following that, they are infected with Herpes Simplex Virus 1 (HSV-1) and further cultured for another 3 days to assess the impact of the virus on Alzheimer's disease (AD) pathology.

2.3 Immunofluorescence and Antibody Staining

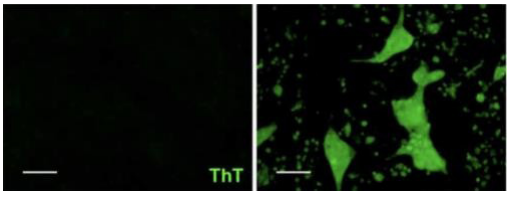

Fig.1 Presence of microglia increases Abdeposition after HSV-1 infection.[3]

Staining with Thioflavin T (ThT) is used for labeling Aβ fibrils, and the extent of microglia is checked for its contribution in Aβ deposition.

2.4 Inhibition of Cytokine Expression:

Fig.2 shRNA Sequence and Construct shRNA Expression Vector[3]

2.5 Design and Construction of shRNA Sequence

To examine the potential of specific cytokines in the pathogenesis of Alzheimer's disease, we designed an experiment with IL-6 as a prototype cytokine.At first, the shRNA sequence was designed according to the mRNA of the IL-6 gene.These sequences were chosen to selectively suppress the gene expression of IL-6 in microglial cells.

2.6 Construction of shRNA Expression Vector

The designed shRNA sequence was then obtained and inserted into an expression vector that will allow transfection of microglial cells. The shRNA expression vector was created using conventional molecular biology techniques to place the shRNA sequence under the control of an appropriate promoter in order to achieve the highest possible expression in microglial cells.

2.7 Introduction of shRNA into Microglia

To investigate the involvement of certain cytokines in Alzheimer's disease, we proposed an experiment that focused on the IL-6 cytokine. It was first necessary to design a shRNA sequence from the IL-6 gene's mRNA. This sequence was particularly selected in order to selectively knock down and inhibit the IL-6 production in microglial cells. Verification of IL-6 Knockdown by Western Blot
To further prove the knockdown of IL-6 expression, the proteins of the microglial cells that were transfected with the IL-6 siRNA were further analyzed, and the levels of IL-6 protein were determined through Western blot analysis. A marked reduction of IL-6 protein levels in treated cells as compared to the control cells validated the knockdown of IL-6 expression through shRNA.

2.8 Repetition of Aβ Distribution Experiment

Having thus validated the IL-6 knockdown, the prior experiments on Aβ distribution within the microglia-containing brain organoids were repeated. The changes in Aβ deposition and distribution were analyzed by the Thioflavin T (ThT) staining method, which helps to determine the impact of IL-6 inhibition on the amyloid load.

2.9 Predicted Experimental Results

Interleukin-6 (IL-6) has the potential to modulate the generation and clustering of Amyloid-beta (A-beta). Upon suppression of IL-6 expression, neuroinflammation is mitigated, resulting in a decline in A-beta aggregation and a subsequent reduction in the monitored fluorescent signal. Nevertheless, despite this reduction, A-beta aggregation remains discernible when compared to the HBO model devoid of microglia. The results are depicted in the accompanying figures. Figure a illustrates HSV-1 infected HBO without microglia, whereas figure b exhibits the distribution pattern of Aβ in HBO with microglia. In figure c, a notable decrease in the fluorescent signal is observed

upon inhibiting the expression of IL-6.

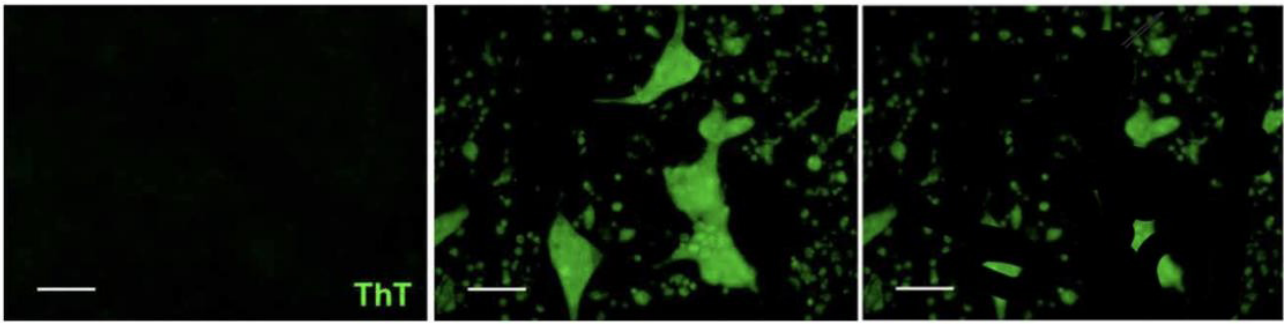

Fig.3 Predicted result of Aβ distribution after cytokines inhibition. Aβdistribution (a) in HSV-1 infected HBO; (b) in HSV-1 infected MC-HBO; (c) in HSV-1 vector infected MC-HBO, with IL-6 knockout.[3]

In a similar vein, we endeavored to devise a range of shRNAs aimed at suppressing the expression of various cytokines, while also anticipating the outcomes of our experiments. Upon inhibiting pro-inflammatory cytokines, we observed a marked attenuation in the Abeta signal along with a decrease in aggregation. Conversely, when Anti-inflammatory cytokines were targeted for inhibition, the A-beta signal intensified and aggregation levels augmented.

Table 1. After inhibiting inflammatory factors expression, Aβ aggregation changes in the HSV-1 infected MC-HBO[3]

	Pro-inflammatory cytokines		Anti-inflammatory cytokines	
Inhibited cytokines	TNF-α	IL-6	IL-10	IL-4
Signal in MC-HBO	decrease	decrease	increase	increase

3. Conclusion

Alzheimer's disease (AD) is a progressive neurological disorder that severely impairs cognitive functions, including memory and language abilities. The etiology of AD remains complex, but growing evidence suggests a potential causal link between herpes simplex virus type 1 (HSV-1) infection and AD. Key pathological hallmarks of AD include the accumulation of highly phosphorylated tau proteins and the formation of amyloid-β (Aβ) plaques, which contribute to the degeneration of synapses and neurons. Neuroinflammation plays a critical role in AD pathogenesis, with microglia, the primary immune cells of the central nervous system (CNS), adopting reactive states in response to viral challenges and other pathological stimuli.

This study introduces a microglia-containing 3D human brain organoid (MC-HBO) model to investigate the role of HSV-1 in AD pathogenesis. Specifically, the model explores the influence of cytokine modulation—both pro-inflammatory (e.g., TNF-α, IL-6) and anti-inflammatory (e.g., IL-10, IL-4)—on Aβ aggregation. The findings indicate that the inhibition of pro-inflammatory cytokines leads to a reduction in Aβ aggregation, whereas the suppression of anti-inflammatory cytokines results in an increase. These insights suggest that targeted intervention in cytokine signaling pathways may present a novel therapeutic strategy for the attenuation of AD's progression.

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