ISSN 2959-409X

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

Zhengtao Li

International Department, Qingdao No.2 Middle School, Qingdao, 266000, China
18863989812@163.com

Abstract:

As a progressive neurodegenerative disorder, Alzheimer's disease (AD) can result in significant memory loss and cognitive deterioration. Several recent studies show that herpes simplex virus type I (HSV-1) is closely related to AD pathology. Microglia, the primary immune cells in the central nervous system, are also shown to play an important role in AD. In this study, we developed a microglia-containing 3-dimensional (3D) human brain organoid from human-induced pluripotent stem cells (hiPSCs) to study the relationship between microglia and HSV-1-induced AD phenotypes. We found that microglia increased the generation of amyloid-β protein (Aβ) and neurofibrillary tangles (NFTs), contributed to neural loss, and enhanced gliosis and neuroinflammation. We also employed shRNA vectors to inhibit certain cytokines related to neuroinflammation and examined the impact on Aβ aggregation. This model can facilitate future research on the treatment of AD regarding microglia and HSV-1.

Keywords: Human brain organoids, Microglia, Alzheimer's disease, hiPSCs.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with symptoms including severe cognitive deterioration, short-term memory loss, speech impairment, and eventually an inability to carry out daily activities [1]. Approximately 50 million people worldwide suffer from AD, and according to the World Alzheimer Report, this number is expected to increase to 152 million in 2050 [2]. Despite the current progress on understanding typical phenotypes of AD, including amyloid plaques, neurofibrillary tangles (NFTs), neuroinflammation, and gliosis, the cause of this devastating disease, unfortunately, remains elusive [3].

In recent years, mounting evidence indicates that the cause of AD is strongly associated with virus infection, including herpes simplex virus type I (HSV-1). HSV-1 is a widespread human specific virus that can establish lifelong infection, accounting for a heavy burden on the patients as well as their family members in both life and economy [4]. Since animal models, like mice and rats, lack human genetic background and significant differences exist between human's and animals' nervous system, they cannot accurately replicate the pathological features of AD. Thus, an in-vitro human brain organoid (HBO) is strongly needed. Microglia, which constitute 10-15% of total cells in the brain, are the primary immune cells in the central nervous system (CNS) [5]. They involve in neuronal development, uptake of aggregated protein, and synaptogenesis [6-9]. Given that microglia play a crucial role in AD pathology, adding them to a human brain organoid can make it more realistic.

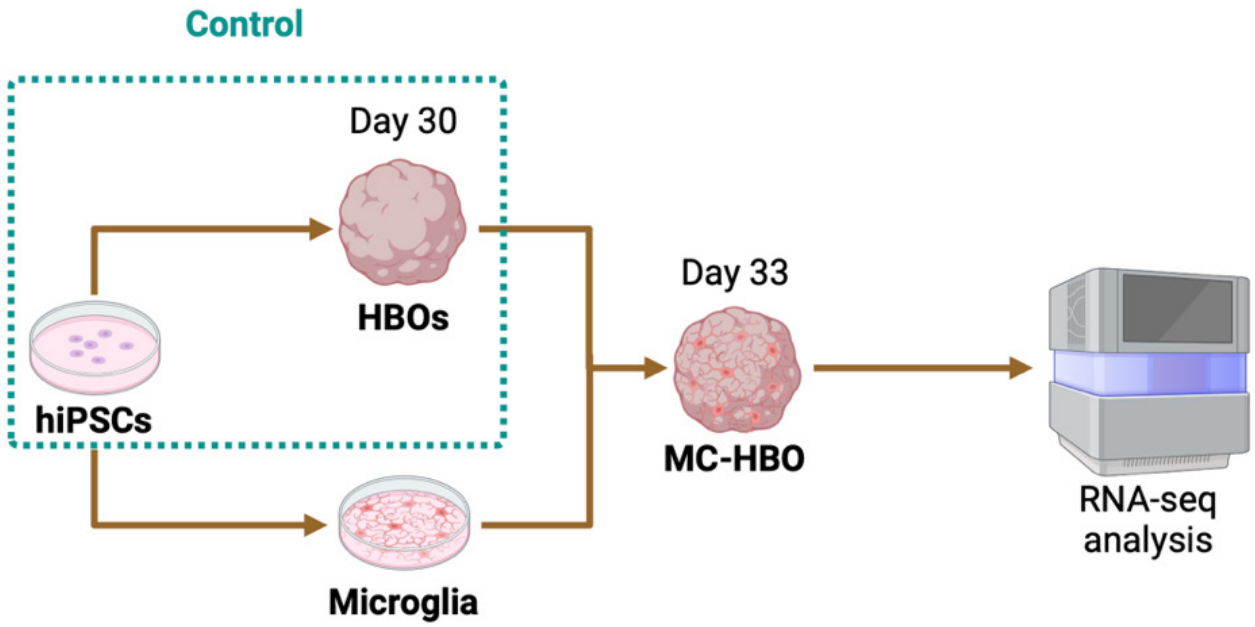

Figure 1. Generation of MC-HBO and the experimental design

In this study, we aim to examine the impact of microglia in the HSV-1-infected HBO on AD pathology. Following the protocol proposed by Bejoy et al., we generated a microglia-containing 3D human brain organoid (MC-HBO) to study the critical roles of microglia in the HSV-1-induced AD. We used human induced pluripotent stem cells (hiPSCs) to generate HBO and microglia separately, and, on day 33, they were mixed and formed an MC-HBO [10,11]. Then, we performed single cell RNA sequencing (scRNA-seq) for microglia characterization (Figure 1). In the subsequent experiments, an HBO without microglia was set as a control compared with the MC-HBO. Both organoids were cultured for 33 days and then inoculated with HSV-1 for 3 days, and we detected the effects of microglia on the typical AD features. We also used shRNA to inhibit the expression of certain cytokines (e.g., TNF-α, IL-6, IL-4, and IL-10) to determine the impact of them on Aβ aggregation. Overall, we developed an effective MC-HBO mimicking HSV-1 infection and AD pathology, enabling the examination of microglia's significant roles in AD pathology. We expected to provide this model as a platform facilitating further explorations on the correlation between microglia and AD and research on potential therapy targeting HSV-1 on AD.

2. Results

2.1 The presence of microglia in the human brain organoid increased Aβ deposition

At first, we determined the effects of microglia on amyloid-β protein (Aβ) generation. The MC-HBO and the control were cultured for 33 days and infected with HSV-1 for 3 days. Thioflavin T (ThT), a fluorescent dye that produces increased fluorescence when binding to amyloid fibrils, was used to label amyloid fibrils in the HBOs [12]. We found that the organoid containing microglia was more positive for ThT than the control, suggesting that there was increased Aβ deposition with the presence of microglia.

2.2 Microglia engulfed Aβ in the human brain organoid

To further investigate the correlation between microglia and Aβ deposition, we performed a subsequent experiment. ThT was used to label Aβ. To recognize microglia, transmembrane protein 119 (TMEM119), which is exclusively expressed in microglia at a high level in human brains, was subject to immunostaining with antibodies against them [13,14]. We found that there was ThT in microglia (i.e., there is color overlap), indicating that microglia engulfed Aβ in the HBO.

2.3 The presence of microglia enhanced the generation of NFTs, which are caused by hyperphosphorylation of protein tau

To determine whether the presence of microglia has influence on the generation of NFTs, a typical AD phenotype caused by aggregation of protein tau, we stained hyperphosphorylated tau using phospho-tau antibodies in the HBOs [15]. We found elevated NFTs in the MC-HBO compared with the control.

2.4 Microglia-containing human brain or-

ganoid exhibited a more obvious reduce in its size, suggesting neural loss

In addition, we aimed to demonstrate if microglia are also related to another typical AD feature, substantial neural loss. Neuron-associated proteins neuronal class III β-tubulin (TUJ1) and microtubule-associated protein 2 (MAP2) were visualized by immunofluorescence in the HBOs. We found significantly lower presence of these proteins in the HBO with microglia than the HBO without microglia, indicating a more substantial neural loss in the HBO due to microglia.

2.5 The presence of microglia resulted in increased gliosis and neuroinflammation

We also demonstrated the relationship between microglia and other HSV-1-induced AD phenotypes, such as gliosis and neuroinflammation. After 33 days of culture, the MC-HBO and the control were exposed to HSV-1 for 3 days. First, we used antibodies against glial fibrillary acidic protein (GFAP)—a glia marker—to indicate glia in the HBOs. We found that there was more GFAP in the MC-HBO than the HBO that did not contain microglia, implicating increased gliosis in the HBO with the presence of microglia.

Furthermore, we assayed markers of neuroinflammation. Tumor necrosis factor-α (TNF-α), one of the main pro-inflammatory cytokines, is involved in many neurodegenerative diseases, and interleukin-6 (IL-6) is another pro-inflammatory cytokine [16]. We examined the expression of these factors before and after HSV-1 infection using PCR (Figure 2A, B). The expression of anti-inflammatory cytokines, including interleukin-4 (IL-4) and interleukin-10 (IL-10), were also assayed before and after infection (Figure 2C, D). We demonstrated that the expression of the pro-inflammatory cytokines in the MC-HBO significantly increased after infection, and the expression of the anti-inflammatory cytokines decreased after infection, which revealed increased neuroinflammation. However, the expression of the cytokines in the HBO without microglia showed no statistically significant difference before and after HSV-1 infection.

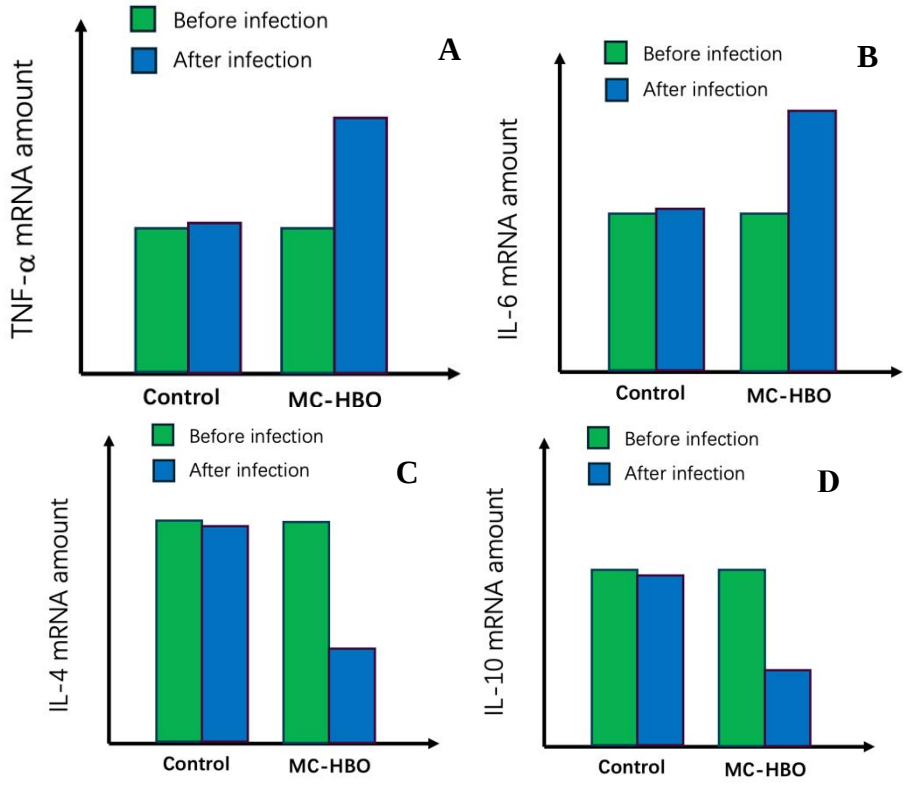

Figure 2. The presence of microglia resulted in increased neuroinflammation. (A, B) The mRNA amount of pro-inflammatory cytokines was examined by PCR in the HBOs. (C, D) The mRNA amount of anti-inflammatory cytokines was monitored by PCR before and after HSV-1 infection.

2.6 Inhibition of inflammatory factors' expression resulted in changes in Aβ aggregation in the MC-HBO

To further characterize the impact of the cytokines mentioned before (TNF-α, IL-6, IL-4, and IL-10) in the MC-HBO, we used shRNA to inhibit the expression of certain cytokines. We designed an shRNA sequence based on the mRNA sequence of the target gene. Then, we constructed an shRNA expression vector and introduced it into microglia cells (Figure 3). Finally, we used western blot to verify the cytokines' level to confirm that the shRNA inhibited the expression of these cytokines successfully. ThT was again used to label amyloid fibrils. We found that the inhibition of the pro-inflammatory cytokines down regulated Aβ aggregation in the MC-HBO; in contrast, inhibiting the anti-inflammatory cytokines up regulated Aβ aggregation (Table 1).

Figure 3. An shRNA vector used to inhibit certain cytokines

Table 1. Predicted changes in Aβ aggregation in MC-HBO after inhibiting certain cytokines

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

3. DISCUSSION

In this study, we developed a microglia-containing human brain organoid to investigate the crucial roles of microglia in HSV-1-induced AD. We found that microglia were strongly related to many AD phenotypes. The presence of microglia enhanced the generation of Aβ and NFTs and increased gliosis and neuroinflammation, indicating the potential relationship between microglia and AD pathology. It also suggested the potential interaction between microglia and HSV-1, which might help discover more effective medicine for AD. Our organoid containing microglia was close to reality and can be used to study other AD treatments regarding microglia and HSV-1. However, our model had limitations. The protocol we used was time-consuming and required multiple proteins to induce microglia and HBO from hiPSCs. In the future, we will explore the underlying mechanisms for the up regulated AD phenotypes by microglia.

References

[1] Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimers Dement. 2016 Apr;12(4):459-509. doi: 10.1016/j.jalz.2016.03.001. PMID: 27570871.

[2] Dallemagne P, Rochais C. Facing the complexity of Alzheimer's disease. Future Med Chem. 2020 Feb;12(3):175-177. doi: 10.4155/fmc-2019-0310. Epub 2019 Nov 21. PMID: 31747794.

[3] Cairns DM, Rouleau N, Parker RN, Walsh KG, Gehrke L, Kaplan DL. A 3D human brain-like tissue model of herpes-induced Alzheimer's disease. Sci Adv. 2020 May 6;6(19):eaay8828. doi: 10.1126/sciadv.aay8828. PMID: 32494701; PMCID: PMC7202879.

[4] Qiao H, Zhao W, Guo M, Zhu L, Chen T, Wang J, Xu X, Zhang Z, Wu Y, Chen P. Cerebral Organoids for Modeling of HSV-1-Induced-Amyloid β Associated Neuropathology and Phenotypic Rescue. Int J Mol Sci. 2022 May 26;23(11):5981. doi: 10.3390/ijms23115981. PMID: 35682661; PMCID: PMC9181143.

[5] Harry GJ. Microglia during development and aging. Pharmacol Ther. 2013 Sep;139(3):313-26. doi: 10.1016/j.pharmthera.2013.04.013. Epub 2013 Apr 30. PMID: 23644076; PMCID: PMC3737416.

[6] Hong Y, Dong X, Chang L, Xie C, Chang M, Aguilar JS, Lin J, Lin J, Li QQ. Microglia-containing cerebral organoids derived from induced pluripotent stem cells for the study of neurological

diseases. iScience. 2023 Feb 24;26(3):106267. doi: 10.1016/j.isci.2023.106267. PMID: 36936782; PMCID: PMC10014280.

[7] Cowan M, Petri WA Jr. Microglia: Immune Regulators of Neurodevelopment. Front Immunol. 2018 Nov 7;9:2576. doi: 10.3389/fimmu.2018.02576. PMID: 30464763; PMCID: PMC6234957.

[8] Nayak D, Roth TL, McGavern DB. Microglia development and function. Annu Rev Immunol. 2014;32:367-402. doi: 10.1146/annurev-immunol-032713-120240. Epub 2014 Jan 22. PMID: 24471431; PMCID: PMC5001846.

[9] Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT. Synaptic pruning by microglia is necessary for normal brain development. Science. 2011 Sep 9;333(6048):1456-8. doi: 10.1126/science.1202529. Epub 2011 Jul 21. PMID: 21778362.

[10] Bejoy J, Yuan X, Song L, Hua T, Jeske R, Sart S, Sang QA, Li Y. Genomics Analysis of Metabolic Pathways of Human Stem Cell-Derived Microglia-Like Cells and the Integrated Cortical Spheroids. Stem Cells Int. 2019 Nov 18;2019:2382534. doi: 10.1155/2019/2382534. PMID: 31827525; PMCID: PMC6885849.

[11] Zhang W, Jiang J, Xu Z, Yan H, Tang B, Liu C, Chen C, Meng Q. Microglia-containing human brain organoids for the study of brain development and pathology. Mol Psychiatry. 2023 Jan;28(1):96-107. doi: 10.1038/s41380-022-01892-1. Epub 2022 Dec 6. PMID: 36474001; PMCID: PMC9734443.

[12] Biancalana M, Koide S. Molecular mechanism of Thioflavin-T binding to amyloid fibrils. Biochim Biophys Acta. 2010 Jul;1804(7):1405-12. doi: 10.1016/j.bbapap.2010.04.001. Epub 2010 Apr 22. PMID: 20399286; PMCID: PMC2880406.

[13] Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A, Weissman IL, Chang EF, Li G, Grant GA, Hayden Gephart MG, Barres BA. New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci U S A. 2016 Mar 22;113(12):E1738-46. doi: 10.1073/pnas.1525528113. Epub 2016 Feb 16. PMID: 26884166; PMCID: PMC4812770.

[14] Satoh J, Kino Y, Asahina N, Takitani M, Miyoshi J, Ishida T, Saito Y. TMEM119 marks a subset of microglia in the human brain. Neuropathology. 2016 Feb;36(1):39-49. doi: 10.1111/neup.12235. Epub 2015 Aug 6. PMID: 26250788.

[15] Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's Disease. Neurotherapeutics. 2022 Jan;19(1):173-185. doi: 10.1007/s13311-021-01146-y. Epub 2021 Nov 2. PMID: 34729690; PMCID: PMC9130398.

[16] Decourt B, Lahiri DK, Sabbagh MN. Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. Curr Alzheimer Res. 2017;14(4):412-425. doi: 10.2174/1567205013666160930110551. PMID: 27697064; PMCID: PMC5328927.