Evaluating the Influence of HCMV Infection on Alzheimer’s Disease Pathology

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BACKGROUND

Alzheimer’s Disease (AD) is a common, debilitating form of dementia typically characterized by a progressive decline in neuronal function that ultimately results in memory loss, unpredictable behavior, and death. Decades of research have uncovered associations between AD and a host of factors (e.g. synaptic deterioration, amyloid plaque/neurofibrillary tangle accumulation, functional deficits, etc.), though little definitive information exists regarding the disease’s underlying mechanisms and pathogenic factors. However, a developing body of literature describes the potential effects that viral infection has on AD pathogenesis and progression.

Several members of the viral family Herpesviridae demonstrate the potential to induce altered cellular phenotypes relevant to AD pathology. Among these herpesviruses is Human Cytomegalovirus (HCMV-5, HCMV). A common pathogen found in 40-70% of the US adult population. Previous studies demonstrate overlap between AD and infection via HCMV-mediated increases in amyloid beta (Ab, in fibrillasts) and associations with increased rates of neurofibrillary tangles (NFTs, phospho-Tau, ptau) in post-mortem brain tissue. Ab and ptau accumulation is known to drive the synaptic dysfunction prevalent in AD. Further, preliminary differential RNAseq data comparing HCMV-infected and mock-treated AD organoids expands upon HCMV’s potential to dysregulate synapses, with infection-dependent downregulation of key synaptic transcripts. Together, these data highlight HCMV’s ability to structurally dysregulate the synaptic compartment and worsen AD pathology. Interestingly, HCMV-infected cerebral organoids demonstrate decreased calcium signaling (baseline calcium; response to KCl) and neurotransmitter release compared to mock conditions.²,³ This contradicts the effects commonly ascribed to AD neurons: increased calcium signaling and hypersensitivity. Considering the existing data, the relationship between AD and HCMV is likely complex, with different aspects of AD pathology being altered independently. Here, we capitalize on the use of patient-derived human induced pluripotent stem cells (iPSCs) to generate 2D forebrain neuron cultures that model the spade-like and familial forms of AD. Then, using the TB40E-eGFP clinical variant of HCMV, we will assess HCMV’s effects on two aspects of AD pathology: ApoE/p tau protein accumulation and synaptic function.

METHODS

Forebrain Neuron Differentiation: Control and AD iPSCs were co-cultured with the STEMdiff™ Forebrain Neuron Differentiation/Maturation kits (STEMCELL Technologies). KHB (STEMCELL, 05151) was used to generate neuronal cultures representative of the human forebrain region.

Calcium Imaging: Calcium Imaging experiments were performed 50 DPI (day post-infection) after infection with each clinical strain of HCMV (TB40/E-eGFP, TB40/E-eGFP + ptau) and mock infection. Data was collected and stored as raw data files using AxioVision (Zeiss). Data analysis was performed using ImageJ.

Viral Infectivity: Free virus was isolated by infecting HCMV-infected and mock-treated AD organoids with the clinical strain TB40/E. Infectivity was assessed via titering experiments. The resulting ratio denotes viral infectivity.

Soluble/Insoluble Western Blots: Soluble/insoluble protein fractions were collected at 7 DPI and 15 DPI. Pellets were lysed and underwent ultracentrifugation steps to separate soluble and insoluble proteins, as described by Santarriaga et. al. Aß1-40 and Aß1-42 values were determined via species-specific ELISA assays (Invitrogen; #KHB3481, #KHB3544). Data is presented as standard amyloid ratio (42/40).

Aß Ratio Analysis: Aß1-40 and Aß1-42 values were determined via species-specific ELISA assays (Invitrogen; #KHB3481, #KHB3544). Data is presented as standard amyloid ratio (42/40).

RESULTS

Functional Implications of HCMV Infection in Forebrain Neurons

Figure 1: Model System Characterization and HCMV Permissivity. (A) Timeline for generating electrophysiologically mature forebrain neurons from patient-derived iPSCs with infected infection schedule and experimental endpoints. (B) Immunofluorescence demonstrates culture system is composed of healthy looking HCMV (TB40E-eGFP) infected (G) and mock-infected (D) forebrain cultures infected with TB40E-eGFP+mCherry characterizing immediate-early transcription. (C) Infection also dampened calcium influx in response to KCl response. (D) Calcium Imaging data demonstrating the detrimental effects of HCMV infection on neuronal capacity for calcium signaling and action potential generation. (E) Aβ accumulation and synaptic function.

HCMV Alters Aβ42 and pTau Amounts in Forebrain Neurons

Figure 2: HCMV infection affects neuronal cell viability for calcium signaling and action potential generation. (A) In calcium imaging, HCMV-infected clinical strains demonstrated lower basal calcium levels. (B) Infection also dampened calcium influx in response to KCl response. (D) Representative images obtained HCMV-infected and mock-treated forebrain neurons planted on the electrode of an mVAC probe. (E) Example raster plots demonstrating the amount of spontaneous action potential generation in cultures +/- HCMV at 5 DPI and 65 DPI. (F) Timecourse data highlighting the detrimental effects of HCMV infection on neuronal firing, regardless of AD background, from 0 to 65 DPI. *p<0.05; **p<0.01.

REFERENCES/ACKNOWLEDGEMENTS:

1 Lurain et al., J Infect Dis, 2013
2 Sison et al., J Virol. 2019
3 Sun et al., Cell Rep. 2020
4 Santarriaga et al., eLife, 2022

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SUMMARY and CONCLUSIONS

The results of this study provide evidence that HCMV infection may have negative consequences on the development and function of forebrain neurons, which may contribute to the pathogenesis of Alzheimer’s disease. The findings suggest that HCMV infection could enhance the progression of AD, possibly by altering calcium signaling and decreasing neuronal excitability.

Collectively, these data highlight mechanisms whereby active HCMV infection can potentially alter aspects of Alzheimer’s Disease pathogenesis.