Interrogating the effects of human herpesvirus 6 infection on neurons and glia derived from human pluripotent stem cells

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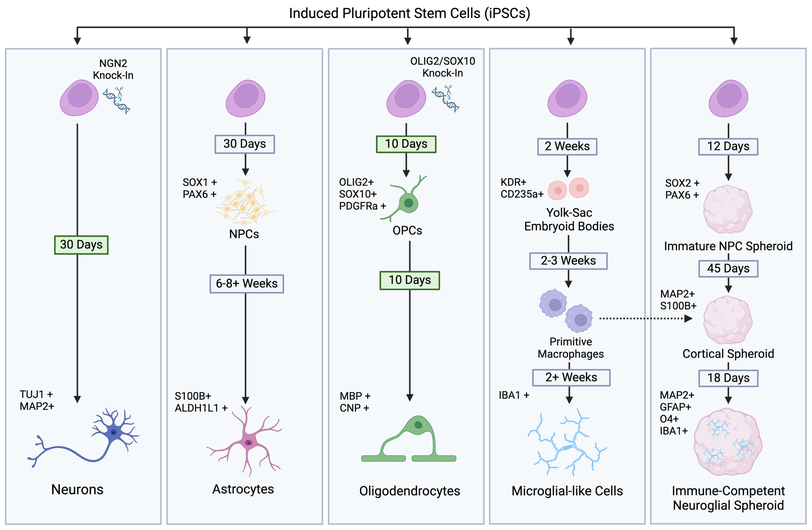
Introduction

Human Herpesvirus 6 (HHV6) is an endemic pathogen, infecting 90% of the world's population at any given time. Acute infection manifests as rash and fever, but can progress to seizures, encephalitis, and even death. Following infection, HHV-6 establishes life-long latency in humans through genomic integration. The consequences of HHV-6 latency are currently unknown but are hypothesized to contribute to the pathogenesis of late-onset adult neurodegenerative disorders including multiple sclerosis and Alzheimer's disease.

- **Understanding the effects of HHV-6 on neurons and glia requires a human model that is robust and scalable.** Animal models do not recapitulate HHV-6 replication dynamics observed in humans, and primary tissue is difficult to obtain on the scale required to investigate viral infections of the brain.
- Human induced pluripotent stem cells (iPSCs) are an unlimited and accessible resource from which to generate large numbers of isogenic neurons and glia, offering a unique opportunity to study HHV-6 infection.
- Using novel optimized differentiation protocols, we can generate each of these cell types in the CNS: neurons, astrocytes, oligodendrocytes, and most importantly the primary immune responder in the brain, microglia.
- Generating each of these cell types in individually, as well as in co-cultures, will enable capture of direct viral effects on each cell type, and allow investigation into the global inflammatory response and cell-cell crosstalk that occurs *in vivo* following infection.

Differentiation of neurons and glia from iPSCs

- Neurons and glia can be derived from stem cells through guided differentiation via small molecule supplementation following developmental timelines, or forced differentiation through iPSCs engineered to express a given protein when induced with an antibiotic that commits them to a particular fate.

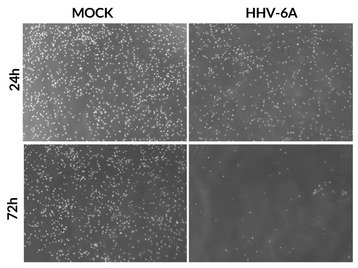


Project Aims

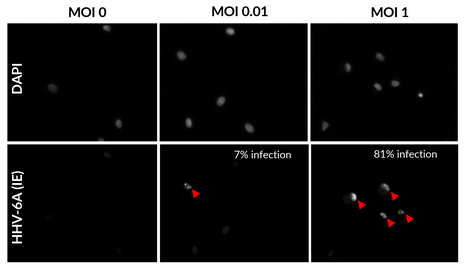
1) **2D Monoculture infections:**
   - Differentiate neurons and glia from iPSCs
   - Infect 2D monocultures of each cell type with HHV-6A
   - Evaluate HHV-6A tropism within the CNS and the subsequent cellular response to viral presence and/or infection with immunofluorescence and RNASeq

2) **3D Co-culture infections:**
   - Generate and infect 3D immune competent cortical spheroids comprised of neurons and glia
   - Analyze the subsequent inflammatory response on the level of single cells

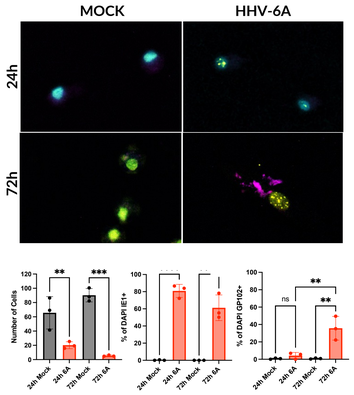
Microglia are acutely vulnerable to infection with HHV-6A in iPSC-derived monocultures



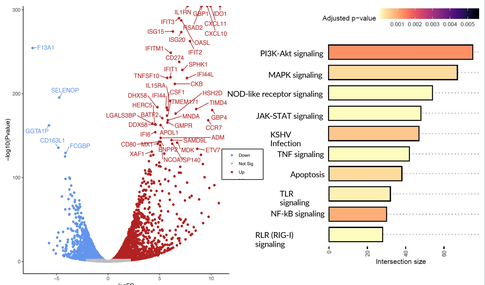
Live imaging of iPSC-derived microglia following infection with HHV-6A. Acute death observed at MOI 3 over a 72h time course relative to an uninfected control.



Immunofluorescent staining for immediate early (IE) HHV-6A viral proteins in microglia 24h post-infection across a range of viral doses. Expression of IE indicating initiation of viral replication observed following infection with the lowest dose attempted, 0.01 infectious particles/cell (MOI).

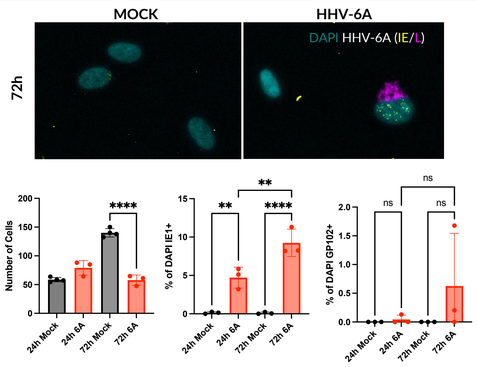


Microglia express early and late HHV-6A viral proteins (IE, L) 72h post-infection, indicating progression through the viral life cycle and active viral replication. Significant cell death as a result is observed at 24h and 72h post-infection. N=3 biological replicates, 9 FOV quantified/well. One-way ANOVA with Tukey's multiple comparisons test. Asterisks denote significance (P<0.05)

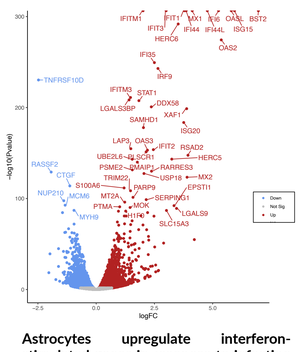


Microglia upregulate genes corresponding to immune activation and viral defense following exposure to HHV-6A. RNASeq performed on samples taken after infection at MOI 1 for 24h. Volcano plot generated in Galaxy showing significantly (p<0.05) upregulated (red) and downregulated (blue) genes, with many interferon-stimulated genes showing significant upregulation (left). GO terms associated with upregulated genes with significant (p<0.05) intersection following analysis with gProfiler (right). Terms include known pathways important to immune response, such as TLR and NF-kB signaling, which are known drivers of inflammation.

Astrocytes decrease proliferation following infection with HHV-6A

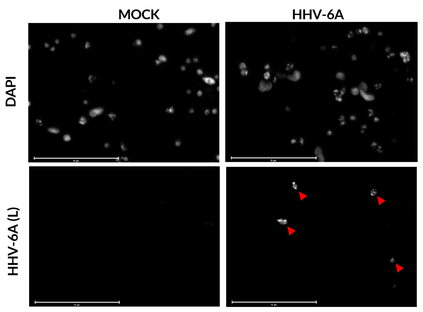


Astrocytes significantly express early (IE1)HHV-6A viral proteins at 24 and 72h, and do not proliferate at the same rate as an uninfected control by 72h. N=3 biological replicates, 9 FOV quantified/well. One-way ANOVA with Tukey's multiple comparisons test. Asterisks denote significance (P<0.05)

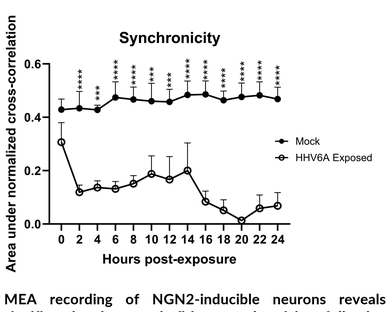


Astrocytes upregulate interferon-stimulated genes in response to infection with HHV-6A at MOI 1 for 24h. Volcano plot generated in Galaxy displaying significantly (p>0.05) upregulated (red) and downregulated (blue) genes.

Neuronal activity is significantly altered following HHV-6A exposure

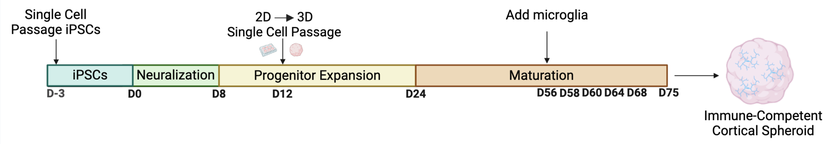


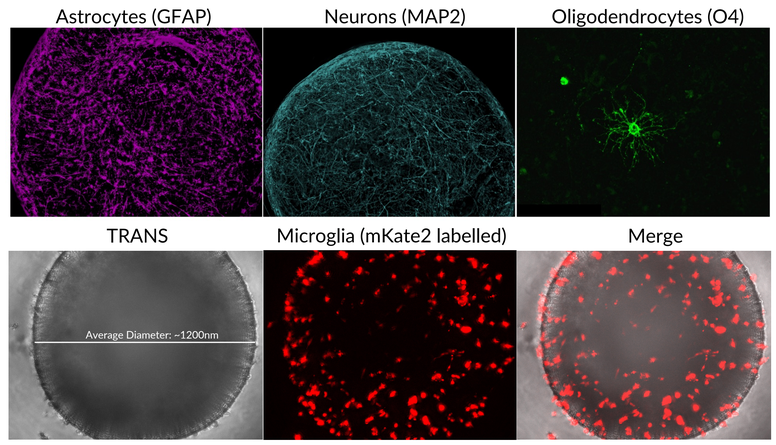
NGN2-inducible neurons infected with HHV-6A at an MOI of 3 over a 72-hour time course express HHV-6A late viral protein. Scale bar= 75um.



MEA recording of NGN2-inducible neurons reveals significantly decreased firing synchronicity following exposure to HHV-6A at MOI 10. N=4 biological replicates, two-way ANOVA, Tukey's multiple comparisons test.

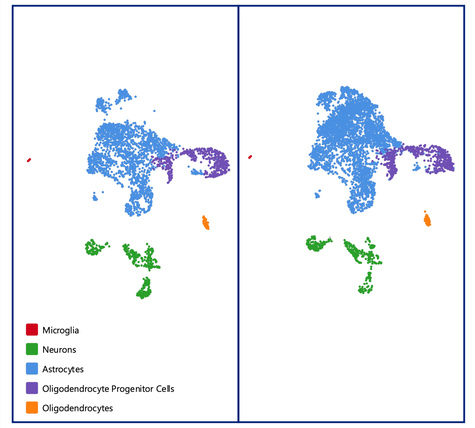
Generation of immune-competent cortical spheroids



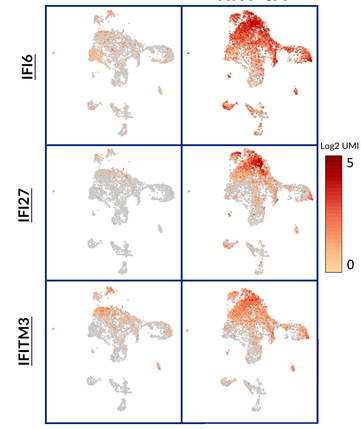


Immune-competent cortical spheroids express markers of neurons and glia after 75 days in culture. Following addition to culture medium, mKate2-labelled microglia efficiently integrate into the tissue.

scRNASeq of immune-competent cortical spheroids infected with HHV-6A



Immune-competent cortical spheroids were infected with HHV-6A at MOI 3, dissociated 72 hours post-infection and processed using the 10x Genomics sample preparation pipeline for single cell analysis. Samples sequenced, and resulting dataset passed through Seurat to create input object for Loupe browser to generate plots. UMAP plots indicate manually annotated cell types present in samples. Immune-competent cortical spheroids displayed gene signatures indicating presence of astrocytes, neurons, microglia, and oligodendrocytes.



Immune-competent cortical spheroids show induction of interferon responses following infection with HHV-6A. Sample UMAP plots of the expression of upregulated genes relative to an uninfected control (scale= log2-transformed UMI counts).

Summary

- Stem cells can be manipulated to model the CNS, thereby enabling investigation of insults such as viral infection
- iPSC-derived neurons and glia are vulnerable to infection by HHV-6A, with infection resulting in acute cytotoxicity in microglia
- Organotypic cortical spheroid co-cultures can also be generated for infection studies, with the addition of microglia rendering cultures immune-competent
- Preliminary analyses of scRNASeq performed on infected co-cultures suggests strong induction of interferon-mediated response to infection