



A human-derived 3D brain organoid model to study JC virus infection

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Abstract

Progressive multifocal leukoencephalopathy (PML) is a frequent neurological complication in immunosuppressed patients. PML is caused by the JC virus (JCV), a neurotropic DNA polyomavirus that infects oligodendrocytes and astrocytes, causing inflammation and demyelination which lead to neurological dysfunction. The pathogenesis of PML is poorly understood due to the lack of in vitro or animal models to study mechanisms of disease as the virus most efficiently infects only human cells. We developed a human-derived brain organotypic system (also called brain organoid) to model JCV infection. The model was developed by using human-induced pluripotent stem cells (iPSC) and culturing them in 3D to generate an organotypic model containing neurons, astrocytes, and oligodendrocytes which recapitulates aspects of the environment of the human brain. We infected the brain organoids with the JCV MAD4 strain or cerebrospinal fluid of a patient with PML. The organoids were assessed for evidence of infection by qPCR, immunofluorescence, and electron microscopy at 1, 2, and 3 weeks post-exposure. JCV infection in both JCV MAD4 strain and PML CSF-exposed brain organoids was confirmed by immunocytochemical studies demonstrating viral antigens and electron microscopy showing virion particles in the nuclear compartment of oligodendrocytes and astrocytes. No evidence of neuronal infection was visualized. Infection was also demonstrated by JCV qPCR in the virus-exposed organoids and their media. In conclusion, the brain organoid model of JCV infection establishes a human model suitable for studying the mechanisms of JCV infection and pathogenesis of PML and may facilitate the exploration of therapeutic approaches.

Keywords JCV · JC virus · Organoid · In vitro model · Human cells · Microphysiological system

Introduction

Progressive multifocal leukoencephalitis (PML) is now a substantial cause of opportunistic viral infection among immunosuppressed people. PML is produced by the JC virus (JCV), a circular DNA polyomavirus that is ubiquitous in the general population, that may establish chronic asymptomatic infections in immunocompetent humans but may produce brain infection in immunosuppressed individuals. JCV can

infect human glial cells and causes massive destruction of oligodendrocytes as well as astrocytic abnormalities that result in demyelination, leading to the development of PML and eventually death (Adang and Berger 2015; Cortese et al. 2021; White and Safak 2016).

JCV was first isolated from the brain tissue of a PML patient in 1971 (Padgett et al. 1971). During the acquired immunodeficiency syndrome (AIDS) epidemic in the 1980s and early 1990s, before the introduction of highly active antiretroviral therapy, PML was reported in 5–10% of people with AIDS. More recently, cases of PML have been reported in association with multiple immunosuppressive and immunomodulatory therapies frequently used to treat rheumatologic and neurological diseases such as infliximab, rituximab, mycophenolate mofetil, and natalizumab (Cortese et al. 2021; Saylor and Venkatesan 2016).

Despite the increasing incidence of PML with the surge of new immunosuppressive agents, the pathogenesis of PML remains poorly understood, and to date, there is no

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effective therapy. JCV infection has been shown to be species-specific, productively infecting human oligodendrocytes and astrocytes but not animal tissue, which has limited the development of *in vitro* and animal models to study the disease. There is an urgent need to study JCV infection in human tissue models that evoke the physiology and homeostasis of the brain to better understand the disease and develop potential therapies. New cell culture models such as 3D cultures and organoids have opened the possibility to study human viral infections in a more physiologically relevant context (Pamies and Hartung 2017). We have previously developed an iPSC-derived 3D human brain model (brain organoid). The brain organoids generated consist of neuronal and glial cell populations in 3D spherical co-culture of approximately 300–350 μm in diameter. The cellular composition is approximately 70% neurons, 20% astrocytes, and 10% oligodendrocytes, with each organoid containing approximately 20,000 cells. The model has exhibited evidence of neuron-neuron and neuron-glia interactions such as spontaneous electrical activity, formation of astrocytic networks, and axonal myelination (Chesnut et al. 2021; Pamies et al. 2017). The model has been successfully used in many applications including modeling virus infections with Zika, dengue, and SARS-CoV-2 (Abreu et al. 2018; Bullen et al. 2020; Chesnut et al. 2021; Kang et al. 2021). Here, we explore the use of the brain organoid as a model to study JCV infection of the human brain.

Methods

Brain organoid generation

The organoid model was generated using a previously described protocol (Pamies et al. 2017). The CRL-2097 line was derived from CCD-1079Sk ATCC® CRL-2097™ fibroblasts purchased from ATCC. First, CCD1079Sk (ATCC® CRL2097™) human fibroblasts were reprogrammed to iPSC with EBV-based vectors and embryoid body formation following a previously described protocol (Wen et al. 2014). Colonies of iPSC were manually picked after 3–6 weeks for further expansion. iPSCs were cultured on irradiated mouse embryonic fibroblasts in human embryonic stem cell (hESC) medium comprising D-MEM/F12 (Invitrogen), 20% Knock-Out™ Serum Replacement (KSR, Invitrogen), 2 mM L-glutamine (Invitrogen), 100 μM MEM NEAA (Invitrogen), 100 μM β -mercaptoethanol (Invitrogen), and 10 ng/ml human basic FGF (bFGF, PeproTech). The medium was changed daily and iPSC lines were passaged using collagenase (Invitrogen, 1 mg/ml in D-MEM/F12 for 1 h at 37 °C). After that, iPSCs were differentiated into neural progenitor cells (NPCs) following Wen's protocol (Wen et al. 2014). NPCs were expanded in poly-L-ornithine and

laminin-coated 175-mm flasks in StemPro® NSC SFM (Life Technologies). Half of the medium was changed every day. Cultures were maintained at 37 °C in an atmosphere of 5% CO_2 .

Then, NPCs were differentiated into brain organoids. Briefly, NPCs were detached mechanically by using a cell scraper and counted using the Countess Automated Cell Counter (Invitrogen). A 2×10^6 cell suspension was plated in 2 ml of medium per well in non-treated 6 well-plates and cultured under constant gyratory shaking (88 rpm, 19 mm orbit). Cells were grown in NPC media for 2 days, and subsequently, the medium was changed to differentiation medium (Neurobasal® electro Medium (Gibco) supplemented with 5% B-27® Electrophysiology (Gibco), 1% glutamax (Gibco), 1% penicillin–streptomycin (Gibco), 0.02 $\mu\text{g}/\text{ml}$ human recombinant GDNF (Gemini), and 0.02 $\mu\text{g}/\text{ml}$ human recombinant BDNF (Gemini). Cultures were maintained at 37 °C, 5% CO_2 under constant gyratory shaking (88 rpm, 19 mm orbit) for up to 8 weeks. The differentiation medium was routinely changed every 2 days.

The resulting glial and neuronal population in the brain organoid were characterized previously (Pamies et al. 2017) by assessing expression of neuronal and glial proteins by flow cytometry, immunocytochemistry, and PCR as well as expression of central nervous system specific genes and microRNAs.

Viral exposure protocol

The brain organoid JCV infection model was generated by exposing brain organoids to the JCV-MAD4 strain or cerebrospinal fluid (CSF) obtained from a subject with PML and high JCV-CSF viral load (JCV⁺CSF) (Fig. 1).

The JCV-MAD4 strain used was obtained from the NINDS, NIH (Bethesda, MD); this strain was isolated originally from the brain of a PML patient (Padgett et al. 1976) and grown and purified per a previously described protocol (Ferenczy et al. 2013). Briefly, the MAD4 isolate of JCV was grown in progenitor-derived astrocytes cells and purified by cellular membrane disruption by 0.25% sodium deoxycholate (Sigma-Aldrich), followed by centrifugation and removal of the cellular debris. The isolate used had a concentration of 1310 HAU/100 μl (1HAU = 10^4 infectious particles) in a suspension of cell culture media (RPMI, Thermo Fisher). The JCV⁺CSF was obtained from the Johns Hopkins Neuroimmunology Biorepository. The CSF was collected from a patient with PML diagnosed by neuroimaging and CSF documentation of JCV by quantitative PCR, which showed a viral load of 118,458 copies/mL.

At 5 weeks of *in vitro* development, brain organoids were exposed to either 40 μl of the JCV-MAD4 isolate (524 HAU) or 40 μl of JCV⁺CSF. Parallel control brain organoids were exposed to 40 μl of virus-free culture media for mock

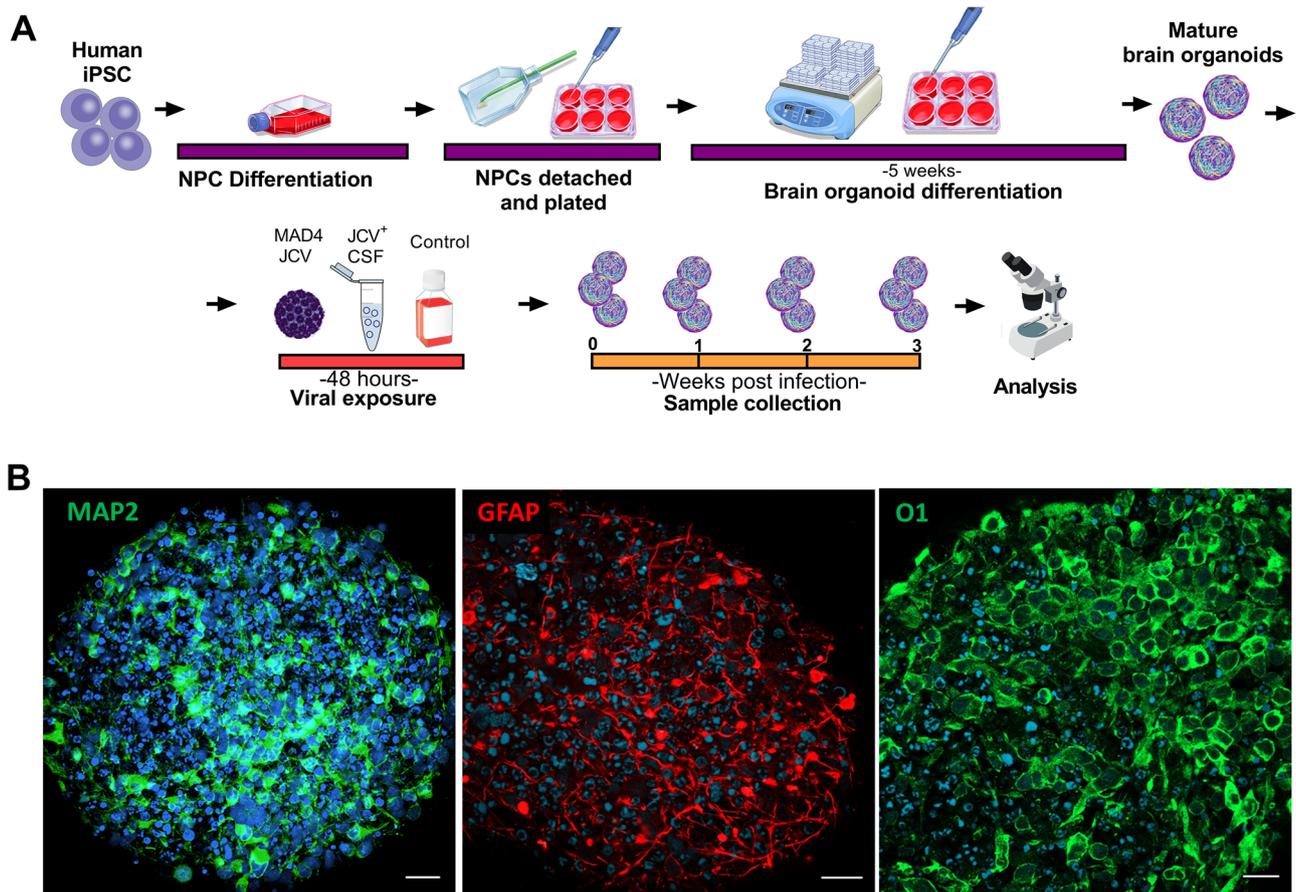


Fig. 1 Experimental design and cell populations in the brain organoid model. **A** Timeline of the brain organoid differentiation and experimental stages. **B** Mature brain organoid cell populations at 5 weeks of in vitro differentiation contain neurons (MAP2⁺), astrocytes (GFAP⁺), and oligodendrocytes (O1⁺ cells). Scale bar: 20 microns

infection, all diluted in 2 mL of the differentiation culture media described above. The brain organoids were kept under constant gyratory shaking in the incubator at 37 °C, 5% CO₂ for 48 h. After 48 h, the virus inoculum was removed, and fresh culture media was added; the brain organoids were kept in culture, and the media was changed every 48 h. The brain organoids were harvested at times 0, 1, 2, and 3 weeks post-exposure, corresponding to 5, 6, 7, and 8 weeks of in vitro differentiation. Immediately after, the organoids were harvested, for each condition, one set was frozen for PCR analysis, and another set was fixed in 4% paraformaldehyde and 3% glutaraldehyde for immunostaining and electron microscopy, respectively (Fig. 1). The experiment was repeated independently 3 times.

Nucleic acid extraction and multiplex qPCR assay

The brain organoid cell pellets were resuspended in normal saline (Quality Biological) at a final volume of 200 µl. DNA was extracted using the QIAamp MinElute Virus Spin kit

(Qiagen) according to the manufacturer's instructions and eluted to a final volume of 50 µl. All eluates were assayed using 20 µl aliquots in duplicate as the template for PCR. TaqMan real-time quantitative PCR was performed using TaqMan PCR chemistry (Applied Biosystems) (Ryschkewitsch et al. 2004). Oligonucleotide primer and probe sets were derived from nucleotide sequences of the non-coding regulatory region (NCRR) of the archetype variant and the large T antigen region of the two major JCV variants, the archetype, and the tandem-repeat/prototype (Ryschkewitsch et al. 2013). A standard curve was set up with each experiment using the JCV MAD1 (Advance Biotechnologies, Inc.) at amounts ranging from 100 pg to 10 ag (attograms) in decreasing 1:10 serial dilutions. A negative control consisting of no template and extraction buffer as template was included with each experiment, along with positive control of a known amount of viral plasmid (JCV MAD1 pM1TC). The real-time reactions were run using the TaqMan Universal PCR Master Mix (Applied Biosystems) with 300 nM of each primer and 200 nM of the probe, in a total volume of 50 µl. The real-time PCR program parameters

Table 1 Primary antibodies used

Antibody	Host	Type	Source	Dilution
VP1-antigen PAB 597	Mouse	Monoclonal	Kindly provided by Dr. Walter Atwood	1:200
SV-40 T antigen	Rabbit	Polyclonal	Santa Cruz	1:200
Olig1	Mouse	Monoclonal	Millipore	1:500
NOGO-A	Rabbit	Polyclonal	Santa Cruz	1:500
GFAP	Rabbit	Polyclonal	Dako	1:500
MAP2	Mouse	Monoclonal	Chemicon	1:1000

were as follows: 50 °C for 2 min; 95 °C for 10 min; then 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The concentration of each experimental sample was calculated using the ABI software and reported as the number of molecular copies/10 µl of the eluted sample. All primers and probes were synthesized to specification by Applied Biosystems (Thermo Fisher Scientific) (Ryschkewitsch et al. 2013).

In addition, the media supernatant for each condition (brain organoids exposed to either JCV-MAD4, JCV⁺CSF, or control) was collected every 48 h after the infection for up to 2 weeks post-infection, before each routine media change. The supernatant was diluted 1:20 in normal saline; qPCR for JCV was performed following the protocol described above. Viral load in the supernatant was reported as the number of molecular copies/mL.

Immunocytochemistry

Brain organoids were fixed in 4% paraformaldehyde, washed three times in PBS, and then incubated for 1 h in blocking solution (5% normal goat serum (NGS) in PBS with 0.4% TritonX). The brain organoids were incubated at 4 °C for 48 h with a combination of primary antibodies (Table 1) in 3% NGS, 0.1% TritonX in PBS. The brain organoids were washed in PBS three times and incubated with a secondary antibody for 1 h in PBS with 3% NGS at room temperature in the dark. Double immunostaining was visualized using the proper combination of secondary antibodies (e.g., goat anti-rabbit secondary antibody conjugated with Alexa 594 and goat anti-mouse secondary antibody conjugated with Alexa 488 (Molecular Probes). Nuclei were counterstained with DAPI (Invitrogen) for 15 min, mounted on slides and coverslipped, and Prolong Gold Antifade Reagent (Molecular Probes); negative controls were processed omitting the primary antibody. Images were taken using a Zeiss LSM 800 confocal microscope.

Immunocytochemistry and confocal imaging techniques were used to determine the presence of JCV infection by examining the expression of viral antigens JCV capsid protein VP-1 and the JCV T antigen in the brain organoids exposed to the different infection and control conditions. Oligodendrocytes were identified by immunostaining with markers such as NOGOA and O1, and astrocytes were identified with antibodies directed to GFAP. Neurons were immunolabelled with MAP-2 and NF antibodies.

Quantification of infection efficiency

Immunolabeling of organoids for identification of infected cells used immunostaining of specific JCV antigens (VP1 and SV-40 T) and co-localization studies with markers for specific cells populations including astrocytes, oligodendrocytes, and neurons (Table 1). Microphotographs obtained at 40× magnification from the three experiments were acquired by confocal imaging. For each time point, at least 15 independent microphotographs from each experiment were obtained. Images were then analyzed with NIH Image J built-in tools for image analysis. We identified the total number of cells based on the number of DAPI stained nuclei quantified using the Analyze particles function of Image J (Schneider et al. 2012). The percent of JCV-infected cells was calculated by dividing the count of JCV VP-1 positive nuclei by the total count of nuclei for a given picture and multiplying by 100.

Electron microscopy

Brain organoids were fixed in 3% glutaraldehyde in PO4 Buffer (EMS, electron microscopy sciences) pH 7.3. Post-fixation was done with 2% osmium for 2 h. The organoids were then stained *en bloc* with 2% uranyl acetate in distilled water for 30 min and subsequently dehydrated in graded ethanol. Embed 812 (EMS) was used as the embedding media. Thin Sects. (100 nm) were cut on a Leica Ultracut UCT Microtome and placed on 200 mesh copper super grids. The grids were stained with uranyl acetate followed by lead citrate, and the sections were examined with a JEM 1400-Plus electron microscope.

Standard protocol approvals, registrations, and patient consents

The acquisition of cell lines and use of patient biological samples followed institutional IRB protocols. Written informed consent was obtained from the patients who provided biological samples. Johns Hopkins University School of Medicine IRB NA_00003551.

Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Morphopathological characterization of the JCV-infection brain organoid model

We observed immunoreactivity for JCV antigens VP-1 and the T antigen in oligodendrocytes and astrocytes (Fig. 2). Most of the JCV VP-1⁺ inclusions were found in enlarged rounded nuclei of O1⁺ and NOGOA⁺ oligodendroglia. The T antigen⁺ inclusions were also found in the nuclear compartment of oligodendroglia, but positive immunoreactivity

was occasionally seen in their cytoplasm. JCV VP-1⁺ inclusions were observed in the nuclei of GFAP⁺ astroglia. These inclusions were observed for the infection with the MAD4 virus as well as in the JCV⁺CSF-exposed brain organoids but not in the organoid controls. No JCV immunoreactivity was identified within neurons immunolabelled with MAP-2 or NF antibodies.

The infection efficiency was measured as the percentage of nuclei expressing the JC VP-1 antigen out of the total number of nuclei at 2 weeks post-infection. The estimated percentage of infected cells out of the total cells in each brain organoid was 2.5% (standard error: 0.3) for the MAD4-exposed brain organoids and 1.4% (standard error: 0.2) for the JCV⁺CSF-exposed brain organoids, respectively, or 8% and 5% of the overall glial population in the MAD4- and JCV⁺CSF-exposed organoids, respectively. No neuronal nuclei were identified with infection.

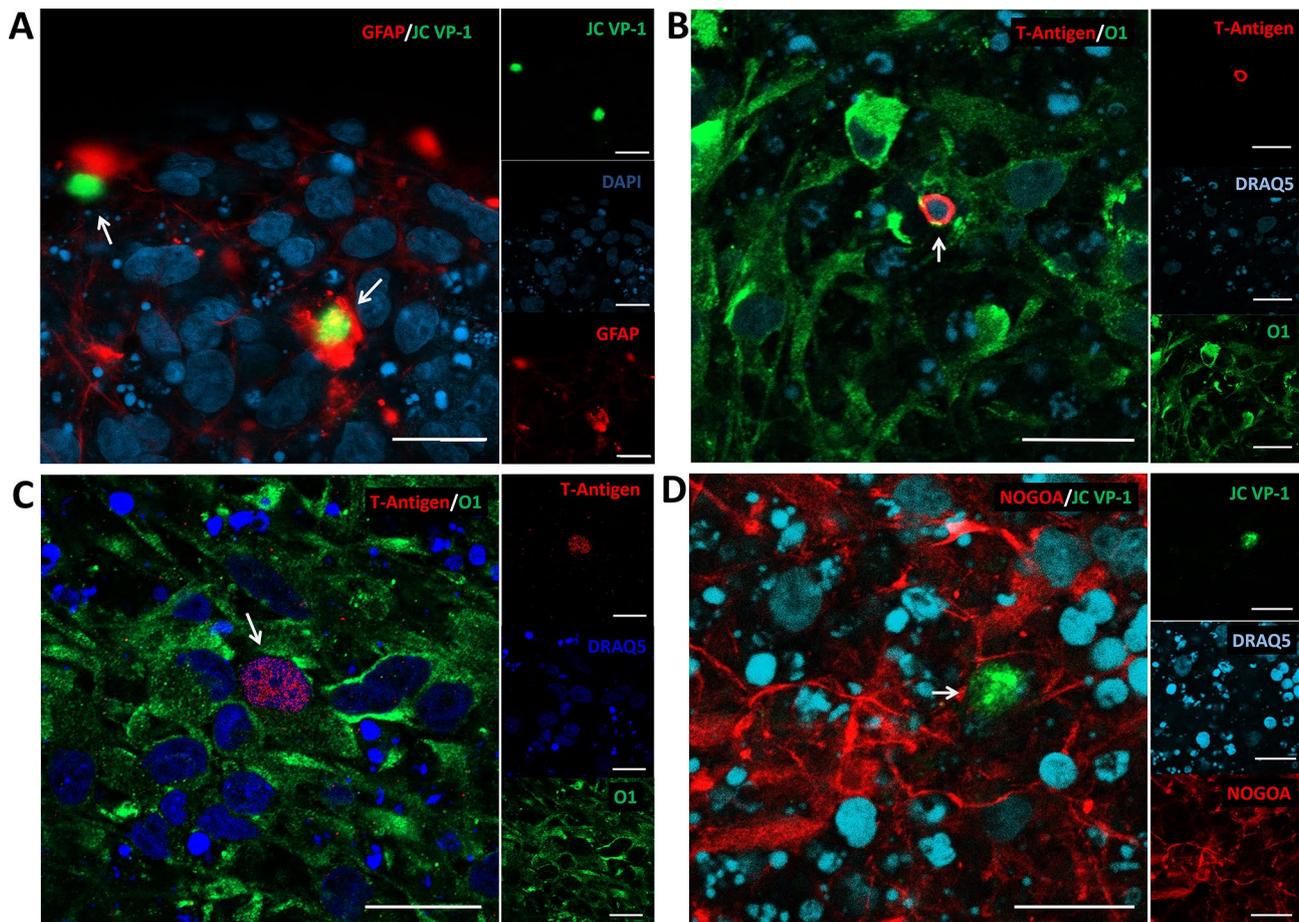


Fig. 2 Immunocytochemical confocal imaging micropathology of the JCV-infected brain organoids. JCV MAD4 virus-exposed brain organoids at 2 weeks post-infection revealed nuclear VP-1 capsid protein (PAB-597 antibody) positive inclusions in GFAP⁺ astrocytes (A) as well as perinuclear (B) and nuclear (C) inclusions expressing T-antigen (SV-40 antibody) in O1⁺ oligodendrocytes. Brain organoids exposed to JCV⁺CSF also revealed VP-1⁺ nuclear inclusions in oligodendroglia (D). Scale bar: 20 microns

Electron microscopy confirmed the presence of JC viral particles in the nuclei of brain organoid cells

To further confirm the presence of JCV in the nuclear inclusions in the brain organoids, we evaluated the brain organoid cell pellets by electron microscopy. We identified electron-dense particles that measured 45 nm in diameter consistent with JCV virion particles in glial-like cells in the MAD4 and JCV⁺CSF-exposed organoids but not in the controls (Fig. 3). The virion particles were arranged in clusters and seen in the nuclear compartment in most cases. Rarely, clusters of viral particles were also observed in the cytoplasm and cellular debris of necrotic cells.

Polymerase chain reaction demonstrated JCV infection in the brain organoid model

The JCV DNA in the different brain organoid conditions (JCV-MAD4, JCV⁺CSF, and mock infection) was quantified by polymerase chain reaction (qPCR) amplification of DNA extracted from the brain organoid cell pellets at time points 0, 1, 2, and 3 weeks post-infection. Quantitative analysis revealed the presence of JCV DNA in the MAD4-exposed brain organoid in all the experimental time points (Fig. 4). The brain organoid exposed to JCV⁺CSF were also positive by qPCR in all experiments and during the 3 weeks post-infection with a lower number of copies per ml than the

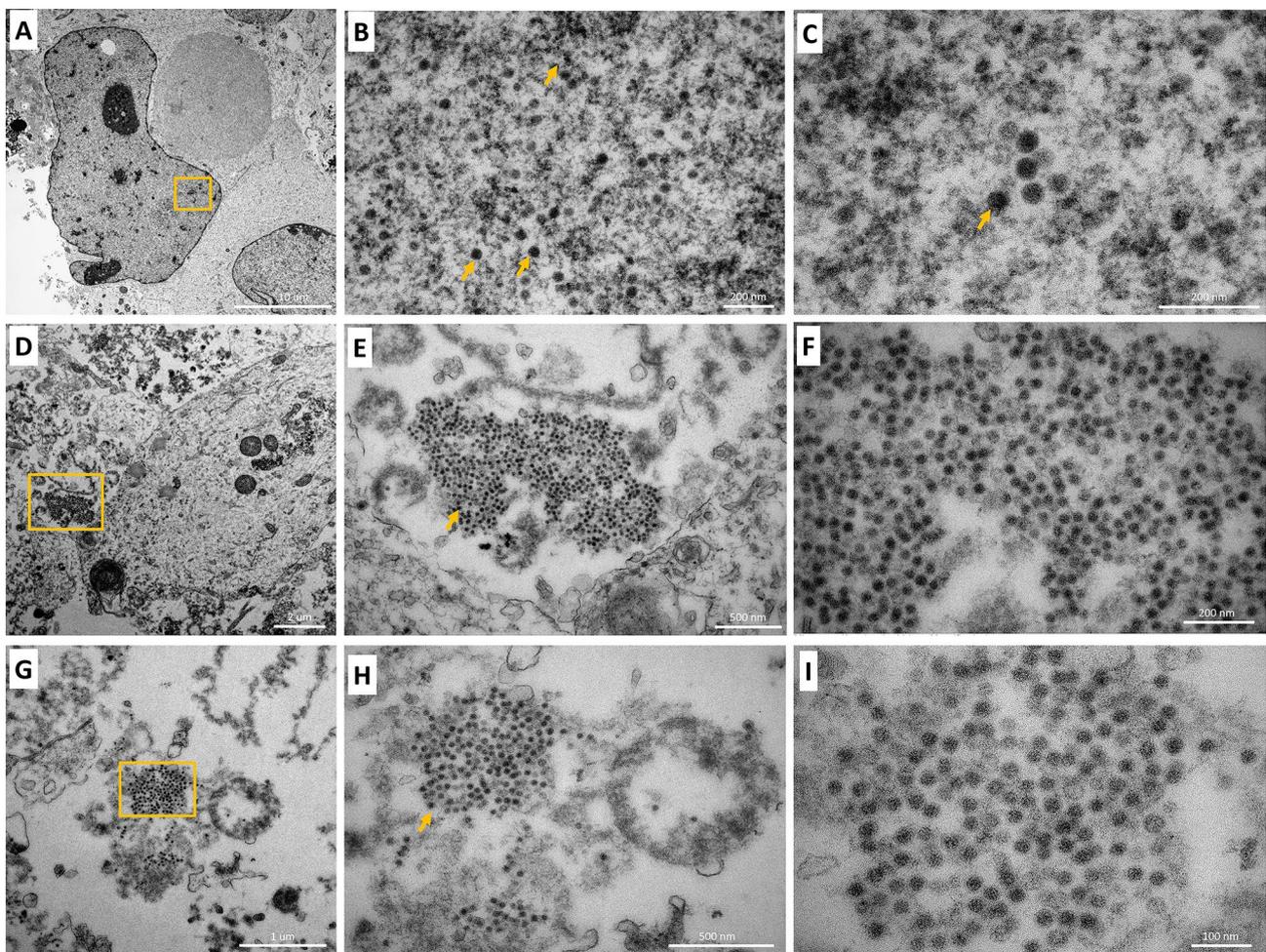


Fig. 3 Electron microscopy imaging of JCV-infected brain organoids. (A) At 2 weeks post-infection, JCV-MAD4-exposed brain organoids exhibited nuclear inclusions with 45-nm icosahedral viral particles, (B) and (C) showing a close-up of the area in the yellow rectangle. Inclusion clusters were also seen in the cytoplasm of some cells (D), close-up (E), (F) and necrotic cells (G), close-up (H) and (I). Yellow arrows point to viral particles

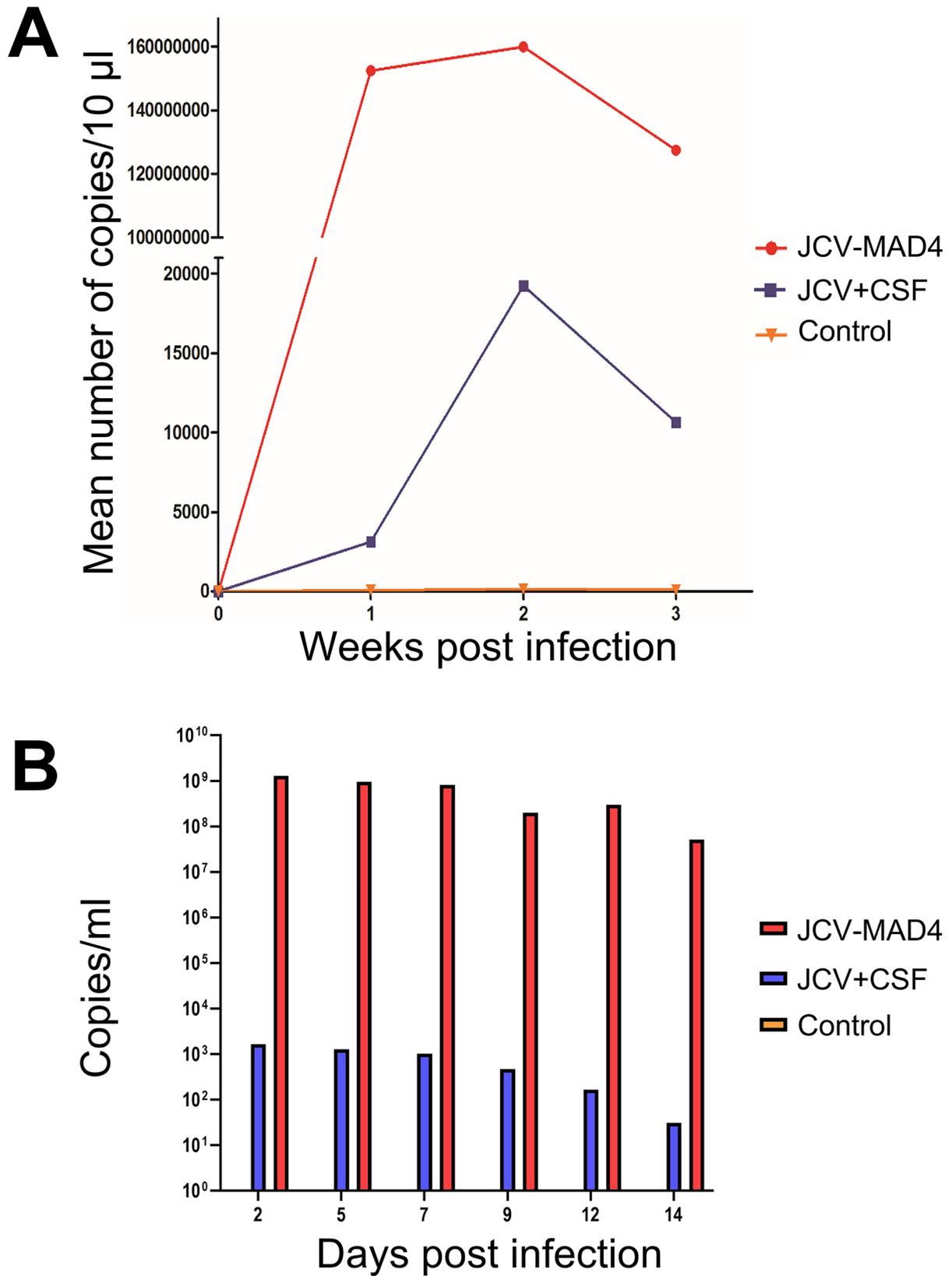


Fig. 4 JCV qPCR results. **(A)** qPCR results of the brain organoid cell pellets expressed as the mean number of JCV copies/10 µL across the 3 experiments for time points 0,1, 2, and 3 weeks post-infection. **(B)** JC viral load in the brain organoid media over time expressed as the number of JCV copies/mL at days 2, 5, 7, 9, 12, and 14 post-infection

MAD4 virus-exposed brain organoid. At the 3-week time point, we observed a decline in the number of copies for both conditions likely explained by cell death because of the infection. In addition, qPCR analysis was performed in the media supernatant of the brain organoids every 48 h for up to 2 weeks after exposure to the different conditions. Figure 4 demonstrates an increase of JCV DNA in the cell media of the brain organoids exposed to JCV-MAD4 and a lesser extent JCV⁺CSF, mainly in the first week post-infection after which the virus copy number decreased.

Discussion

JCV is the causal agent of PML, an often-fatal neurological disease characterized by multifocal lesions in the white matter causing demyelination and cell death; it occurs typically in immunosuppressed individuals (Adang and Berger 2015; Cortese et al. 2021). While most of the population has been exposed to JCV (Taguchi et al. 1982), the development of PML remains rare even among immunosuppressed patients. It is not clear why PML seems more common in patients taking certain immunomodulatory medications than in others or what drives viral entry into the central nervous system. Viral and host factors may play roles in the development of PML beyond the immunosuppressed state, and models that allow the understanding of those roles are critical in the development of effective therapies. JCV selective productive infection of human cells has limited the establishment of *in vitro* and animal models to study the disease, which in turn has resulted in a lack of mechanistic understanding of PML and therapeutic options. Here, we have established a human brain organoid model to study JCV infection; the infection in the model was demonstrated by PCR and immunohistochemistry and confirmed by electron microscopy in astrocytes and oligodendrocytes.

In the past, inoculation of JCV into mice or hamsters failed to produce demyelination but resulted in tumor formation (Barth et al. 2016). Transgenic mice expressing constitutively the JCV T antigen have demonstrated variable phenotypes that do not recapitulate PML (Barth et al. 2016). The infection with SV40, a polyomavirus that has 70% sequence homology with JCV, in the AIDS simian model can cause demyelination, which is analogous to PML in HIV-infected individuals (Horvath et al. 1992). While this model is promising to study features of the disease, it may not be the best platform for screening therapeutic compounds on a large scale as only around 2% of the SIV-infected monkeys develop SV40-active infection (Kaliyaperumal et al. 2013). Recently, the human fetal glial cell line SVG (Major et al. 1985), which constitutively expresses the simian SV40 LT antigen, has been used to study JCV infection (Barth et al. 2016), with some success to

screen for therapeutic molecules (Brickelmaier et al. 2009); G144 cells, a glioma derived cell line with an oligodendrocyte precursor-like phenotype, have also been used and proved to support JCV replication (Peterson et al. 2017). However, a modified cell line may not be physiologically relevant as it would not allow studying the interactions with other cell types, which may limit the conclusions about the pathogenesis of the disease. Furthermore, in the astrocytic and precursor cell line models, it is not possible to evaluate the effect on mature oligodendrocytes and myelination, which is the hallmark of the disease. A more recent approach has been the generation of humanized mouse models, developed by implanting human glial progenitor cells into immunodeficient mice (Barth et al. 2016; Kondo et al. 2014). Injection of JCV into the brain of these mice resulted in virus replication mainly in astrocytes and some recapitulation of the PML phenotype with demyelination, although the oligodendrocytes were not frequently infected. That model represents a promising new tool to study JCV and PML but has still a complex protocol that can be very expensive. In addition, the availability of the animals and the ethical issues surrounding animal experimentation would have to be considered.

In the human brain organoid model presented here, we observed viral nuclear inclusions in enlarged rounded nuclei of both oligodendrocytes and astrocytes exposed to the MAD4 virus and the CSF of a patient with PML. While JCV is a DNA virus, we observed occasional cytoplasmic inclusions, which may indicate active viral replication as proteins are transcribed in the cytoplasm and then taken to the nucleus for assembly. Our findings demonstrate that the brain organoids can sustain productive JCV infection for up to 3 weeks post-infection, which should be enough time to screen therapeutic compounds and look at different pathogenic mechanisms. The decline in viral load observed after 3 weeks is likely explained by the death of the target cells due to the initial JCV infection. This brain organoid model has the advantage of containing different human brain cell types in co-culture with meaningful functional interactions. Evaluating interactions between neurons and glial cells in the context of a JCV infection may give additional clues to understand the pathogenesis of the disease. Moreover, we were able to demonstrate productive infection in the brain organoid model exposed to CSF from a patient with PML as reflected by the increase of JCV copy number, finding that may reflect closely the pattern of infectivity of naturally circulating JCV as compared with laboratory-maintained strains such as the MAD4 JC virus.

This model allows for the production of a large number of JCV-infected organoids, in an easy and relatively inexpensive way to have human tissue always ready to use and not to depend on the availability of fetal or animal tissue. The model does not have the ethical issues associated with

the production of animal models or fetal cells as the iPSC used in the brain organoid are derived from fibroblasts of adult human donors. The stable cellular composition and their low size variability make outcome measurement and comparison between different conditions easy. Other models that have tried to use human oligodendrocytes progenitor cells to study JCV have failed as the virus seems to halt oligodendrocyte differentiation and myelin production. Since this organoid model contains mature oligodendrocytes and astrocytes, it allows to study of the infection in more mature human glial cells and not just in progenitor cells. In addition, since the brain organoid is derived from NPCs from reprogrammed human fibroblasts, known not to be permissive to JCV infection (Frisque et al. 1979), the differentiation process which generates glial cells susceptible to infection creates an opportunity to study transcription factors that contribute to JCV infection susceptibility, including factors such as NF1-X (Ravichandran et al. 2006).

The brain organoid model of JCV-infection presented here has some limitations. One of the main limitations of the model includes the lack of an immune system in the organoid, which would limit the use of the model to study immune-viral interactions. Noteworthy, in recent work on Zika and dengue virus, we added the main immunocompetent cell of the brain, i.e., microglia⁹, which represents a major avenue for future research. Another limitation in the quantitative analysis of viral infection is that the concentration of JCV adsorbed was not calculated and viral quantification was not done by hemagglutination assay. However, the use of direct visualization of infected cells using immunolabeling with JCV markers, electron microscopic demonstration of virions, and the increase in JCV copies over time measured by qPCR in the brain organoid pellets and cell media are consistent with a model of viral replication and productive infection.

Overall, these results establish a human-derived brain organoid as a potential model to study pathogenic mechanisms of JC virus infection leading to PML and explore therapeutic compounds.

Author contribution PB, DP, and CP were involved in concept and study design; all authors were involved in data acquisition and analysis; PB, DP, and CP were involved in drafting the manuscript and figures. All authors edited and approved the final version of the manuscript.

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Declarations

Conflict of interest TH, HH, and DP are named inventors on a patent by Johns Hopkins University on the production of mini-brains (also

called BrainSpheres), which is licensed to AxoSim, New Orleans, LA, USA. They consult AxoSim and TH and HTH are shareholders. The rest of the authors have no disclosures.

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