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Pseudovirus mimics cell entry and trafficking of the human polyomavirus JCPyV

Gretchen V. Gee¹, Bethany A. O'Hara¹, Aaron Derdowski¹, and Walter J. Atwood¹

¹Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912

Abstract

The normally asymptomatic human polyomavirus, JCPyV, is the causative agent of a rare but fatal demyelinating disease known as Progressive Multifocal Leukoencephalopathy (PML). Individuals at risk for developing PML include those with AIDS, with other underlying immunosuppressive diseases, and in patients treated with immunomodulatory regimens. Drugs to prevent viral reactivation in the setting of immunosuppression or immunomodulation could be used to sustain lives. Development of such drugs has been impeded by the difficulty of growing and studying the virus. We sought to develop a more efficient method for screening drugs that inhibit viral infection. Pseudovirus models have been developed which may be of use in pharmaceutical research. The use of pseudoviruses as models for viral infection is dependent on them using similar pathways for infection as virus. We screened known inhibitors of viral entry for their ability to block pseudovirus infection. Here we show that the pseudovirus based on the human polyomavirus JCPyV recapitulates virus binding, entry and trafficking. This system can be used for high-throughput screening of antiviral drugs.

Keywords

JC polyomavirus; Progressive multifocal leukoencephalopathy; Pseudovirus; Natalizumab; Virus entry

1.1 Introduction

Among the first human polyomaviruses discovered, JCPyV was isolated from the brain of a Hodgkin's lymphoma patient in the early 1970's (Gardner et al., 1971). Although the virus is common, it is asymptomatic in healthy individuals and virus with archetype regulatory arrangement persists in the kidneys. JCPyV causes the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML). Disease is associated with the prototypical or PML-type virus that is characterized by rearrangements in the regulatory region (Daniel et al., 1996). This rare brain disorder is due to lytic infection of oligodendrocytes, the cells that provide myelin sheathing for neurons. PML is thought to be due to reactivation of latent virus and occurs only in the setting of immunosuppression. Although PML is still considered a defining disease of AIDS, the incidence has decreased

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*Corresponding Author: Walter J. Atwood, PhD., Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, 70 Ship Street, Providence, RI 02903, Phone: 401-863-3116, Fax: 401-863-9653, Walter_Atwood@brown.edu.

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along with advances in highly active antiretroviral therapies (HAART) (Engsig et al., 2009). In the absence of HIV, PML is a rare side effect in patients with other autoimmune diseases such as multiple sclerosis (MS), and hematological disorders, as well as in bone marrow and other transplant recipients (Ferenczy et al., 2012). This risk is increased if the patient's blood tests positive for anti-JCPyV antibodies (FDA, 2012). Recent evidence suggests that PML is becoming more clinically relevant as a widening variety of immunosuppressive treatments are associated with the disease (Schmedt et al., 2012).

Common immunosuppressants such as rituximab, cyclosporine, methotrexate and natalizumab have all been reported as the primary suspected drug in cases of PML. The risk of disease increases over long-term drug treatment. Recent evidence has shown that PML will develop in approximately 1:100 MS patients taking natalizumab, who have been on prior immunosuppressive therapy (Baldwin and Hogg, 2013). Due to lack of alternative MS treatments, when presented with the risks, a small number of patients voluntarily discontinue therapy (van Rossum et al., 2013). Pharmaceutical companies have been forced to stop marketing or selling certain beneficial drugs due to reactivated JCPyV induced PML (FDA, 2006; FDA, 2010; FDA, 2012; Schmedt et al., 2012). The safety of these drugs could potentially be restored if viral reactivation and spread could be inhibited.

Development of drugs to block polyomaviruses has been hampered by the lack of animal models, and the difficulty of polyomavirus propagation (Mahon et al., 2009; Simon et al., 1999; Steiner and Berger, 2012). In addition, the relative inefficiency of these viruses makes drug screening an arduous task. The efficiency is increased in T antigen (Tag) positive cell lines but the presence of this early viral protein requires infection be scored by measuring late viral protein expression. Restrictions on viral DNA replication and transcription due to its weak promoter further inhibits the screening process (Jensen and Major, 2001; Liu and Atwood, 2001). A viral capsid-based model of JCPyV has been developed to study the viral lifecycle, but there is no convenient method of detecting viral infection (Nelson et al., 2012). Pseudovirus models of other polyomaviruses have been developed which have an easier readout and would facilitate the drug screening process (Buck et al., 2005). We have adapted this method to develop a JCPyV pseudovirus model (JCPsV), and have determined that it uses the same pathway as virus to deliver its genome to the nucleus. The JCPsV is easy to grow and provides an efficient model for testing drugs which block binding, entry or viral trafficking.

1.2 Materials & Methods

1.2.1 Plasmids

Codon optimization of the JCPyV VP1, VP2 and VP3 genes were performed according to the National Cancer Institute Center for Cancer Research Lab of Cellular Oncology Technical Files (<http://home.ccr.cancer.gov/LCO/production.asp>) in order for optimal expression in the human derived cell line 293FT (Life Technologies Cat # R700-07). The genes were synthesized by Blue Heron Biotech, LLC, Bothell, WA USA. Sequences were based on the *Polyomaviridae Orthopolyomavirus* strain Mad-1 (NC_001699.1). The VP1 gene was subcloned into the pwP vector in place of the MPyV VP1 gene. The JC VP2 and VP3 genes were subcloned into the ph2p vectors in place of the MPyV genes. (Tolstov et al., 2009) The luciferase reporter vector phGluc (Pastrana et al., 2009) expresses a secreted form of Gaussia luciferase under control of EF1alpha promoter and EGFP under the control of the SV40 promoter. All plasmids were obtained from AddGene (Cambridge MA USA).

1.2.2 Pseudovirus Production

Pseudoviruses were produced by transfection of the VP1, VP2, VP3 and phGluc plasmids into 293FT cells using the FuGENE® 6 Transfection Reagent (Promega Madison, WI USA cat # E2692) in a 5:1:1:1 ratio. As the viral structural proteins are expressed, they assemble into pseudovirus particles and package available plasmid DNA. JCPsV is titered by calculating the number of viral particles that have packaged the phGluc reporter plasmid. A mock virus preparation prepared using the phGluc vector exactly as pseudovirus but substituting a pUC19 empty vector for the viral protein expressing plasmids. This mock virus was used to control for the presence of phGluc or luciferase co-purifying with pseudovirus.

1.2.3 Pseudovirus Purification

Cells were harvested at 48 hours post-transfection by scraping, pelleted and resuspended in Buffer A with protease inhibitors (cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets cat # 04693132001, Roche Applied Science Indianapolis, IN, USA). Cells were lysed by three rounds of freezing and thawing, sonicated and treated with .25% deoxycholic acid at 37°C for 30 minutes. The pH was lowered to 6.0 and the lysates were treated with type V neuraminidase (Sigma Cat# N2876) at 37°C for 1 hour. The pH was then raised to 7.5, calcium chloride was added and the lysate was treated with DNase I (New England BioLabs Ipswich, MA USA cat# M0303S). Pseudoviruses were then purified over an iodixanol gradient, and spun for 3.5 hours at 16°C at 50,000 rpm ($234,000 \times g$) in an SW55ti rotor. The band containing pseudovirus was extracted by syringe. For the mock virus, the fraction corresponding to the position where pseudovirus was identified was extracted. Although plasmids up to 9.4 kbp have been shown to be packaged into the larger VP1-only virus like particles (VLPs), viral vectors usually package DNA close to the size of the genome, and it was assumed that one copy of the phGluc reporter plasmid (4463 bp) would be encapsidated into each infectious JCPsV particle (Fang et al., 2012).

1.2.4 Pseudovirus Titration

To titer the pseudovirus with properly encapsidated phGluc plasmid, the purified pseudovirus was again treated with DNase I to remove any unpackaged plasmid. DNase I was then inactivated by heating to 75° C for 10 minutes. The phGluc reporter plasmid that was protected by the viral capsid from enzymatic degradation was then extracted using the DNeasy Blood & Tissue Kit (Qiagen Valencia, CA USA cat # 69504). The copies of pseudovirus which packaged phGluc were measured using absolute quantification and TaqMan qPCR (Applied Biosystems) to create a standard curve using serial dilutions of phGluc ranging from 15 ng (3 e9 copies) to 1.5 ag (.3 copies) of input DNA. The Ct value for the known amounts of DNA added to the qPCR reaction was plotted against the number of copies of DNA in a scatter plot. The best fit trendline equation from regression analysis was used to calculate the relationship between the Ct value and copies of packaged plasmid. Using this method we could quantify the number of pseudoviruses containing the phGluc reporter plasmid, or genome equivalents (GE) added during each infection.

1.2.5 Cell Lines

293FT cells are derived from human embryonic kidney cells transformed with the SV40 large TAg (Invitrogen Life Technologies), SVGA and SVGR2 cells are subclones of the SV40 origin-defective transformed human glial cell line SVG (Major et al., 1985), SVGR2 cells are resistant to JCPyV infection (Gee et al., 2003), Ntera-2 cells (Pleasure and Lee, 1993) are a pluripotent embryonal carcinoma line, POJ19II (Mandl et al., 1987) are human fetal glial cells transformed with a replication-defective JCPyV. HeLa cells are a human epithelial cell line derived from cervical carcinoma, CV1 and Vero are monkey kidney

epithelial cell lines, C8D1A and C8D30 are mouse astrocytic cell lines, NIH3T3 is a mouse embryonic fibroblast cell line, C6 is a rat glial cell line NRK is a rat kidney epithelial cell line and CHO cells are an epithelial cell line derived from hamster ovaries (ATCC, Manassas, VA).

293FT were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented to contain 10% fetal bovine serum (FBS) (Mediatech, Inc.), 0.1 mM non-essential amino acids (NEAA), 6 mM L-glutamine, 1 mM sodium pyruvate, and 500 mg/ml geneticin. Vero cells are grown in MEM with 5% FBS. All other cells were grown in DMEM or MEM supplemented to contain 10% FBS in a humidified incubator at 37°C and 5% CO₂.

1.2.6 Infections

Cells were seeded 24 hours prior to infection in 96 well dishes at 5000 – 7000 cells per well. Infections were performed by diluting 10⁸ GE/mL in a total volume 35 µL in serum free media and infecting approximately 10,000 cells for two hours at 37°C. Cells were washed and media was replaced with phenol red free media and neutralizing JCPyV antibody diluted 1:1000. Viral infection was measured at 72 hours post-infection by detection of secreted luciferase in the supernatant using the BioLux® *Gaussia* Luciferase Assay Kit (NEB cat # E3300L). Cell toxicity was measured from the same samples using the Cell Titer 96® Aqueous Non-Radioactive Cell Proliferation Assay (MTS) kit (Promega cat # G5421). Metabolically active cells release a product into the supernatant which is measured by absorbance at 490 nm and is directly proportional to the number of cells. Numbers of infected cells were also be measured by counting GFP positive cells per microscope field using microscopy. All experiments were performed in triplicate and bars represent the standard error.

1.2.7 Hemagglutination Assay

Serial two-fold dilutions of iodixanol, PBS, cesium chloride-purified JCPyV, mock PsV and 1 × 10⁷ or 1 × 10⁸ GE of JCPsV were prepared in PBS in a U-bottom 96 well plate (Corning) (dilution range 1:2 to 1:4096). An equivalent volume of human type O negative red blood cells (RBCs) that had been washed with Alsever's buffer (0.1 M D-glucose, 0.027 M sodium citrate, 0.07 M NaCl, pH 6.5) were added to each and plates were gently agitated. Plates were incubated at 4°C for 4 hrs and imaged using a ChemiDoc XRS Imager (Bio-Rad).

1.2.8 Infection Inhibitors/Blockers

Neutralizing antibodies: JCPsV was pretreated for two hours at 37°C with 1:1000 dilution of rabbit-anti-JCPyV, rabbit-anti BKPyV antibody or preimmune serum in serum free media (SFM), before infections. After infections, PsV was removed and replaced with complete media and 1:1000 dilution of antibody. Neuraminidase: Cells were pretreated for 45 minutes at 37°C with PBS (pH5 + Mg and Ca) or 0.8U/100uL neuramidase (Sigma cat# N6514). Clathrin inhibitor: 5 µM chlorpromazine 1 hr (Enzo #ALX-270-171-G001). Endosomal neutralization: 20 mM ammonium chloride (NH₄Cl) (4 hrs) 10 nM monensin (Sigma #M5273) (1 hr), 10 nM bafilomycinA1 (BafA1) (Sigma #B1793) (1 hr) or DMSO (1 hr). ER inhibitors: Thapsigargin (25nM) (Sigma #T9033) and DTT (5 mM) (Sigma) After infections, JCPsV was removed and replaced with complete media and 1:1000 dilution of antibody to remove all unbound pseudovirus. All experiments were performed in triplicate.

1.3.1 Results

Efficient JCPyV propagation occurs in cells derived from the human brain (Kenney et al., 1984; Kim et al., 2000). Viral replication depends on the ability of the virus to successfully

bind and enter cells and deliver its genome to the nucleus. Once in the nucleus, productive infection is further restricted at the level of viral genome by cell-specific factors which drive expression and replication. Earlier work has shown that the JCPyV capsid plays a role in viral tropism (Chen and Atwood, 2002; Elphick et al., 2004). We used this information to develop a model of JCPyV infection that can be used to study the virus and develop therapies.

We first tested JCPsV infection on multiple cell lines to determine whether it behaved like authentic virus or if it would infect cells nonspecifically. SVG cells are a TAg positive cell line derived from primary human fetal glial cells transfected with an origin-defective SV40 mutant (Major et al., 1985). They are the standard cell line used for laboratory cultivation of JCPyV. We used two derivatives of this line, SVGA cells which maintain the characteristics of the parental line and SVGR2 cells, a subset of this line that was derived to be refractory to infection in a manner specific to polyomaviruses (Gee et al., 2003). JCPsV infection was determined by measuring secreted luciferase, expressed from the reporter plasmid, as counted by relative luminescence units (RLU). We show that JCPsV mimics JCPyV in that it is infectious in SVGA cells and infection is inhibited in the SVGR2 line (Figure 1). In addition, JCPsV infected another cell line derived from human fetal glial cells, POJ 19 II (Mandl et al., 1987) as well as Ntera-2 (NT2) cells, a pluripotent embryonal carcinoma cell line with characteristics of committed CNS neuronal precursor cells (Pleasure and Lee, 1993). We detect minimal infection in HeLa cells, a human epithelial line derived from a cervical carcinoma.

The archetype virus is known to persistently replicate in the kidneys and can infect and efficiently replicate even in monkey kidney cells transformed with TAg (Hara et al., 1998). We found modest infection CV1 and Vero cells, TAg transformed kidney epithelial lines derived from monkeys. Consistent with the fact that JCPsV uses a specific entry pathway to deliver the reporter plasmid to the nucleus, infection was undetectable in mouse, rat or hamster cells. (Figure 1) The reporter plasmid pHGluc uses a human promoter to express the luciferase reporter gene but also carries a GFP reporter gene under the control of the SV40 promoter. To rule out that inactivity of the luciferase promoter masked the ability of JCPsV to deliver its genome to the nucleus in cell lines that were negative, we checked promoter activity in each cell line. This was done by transfecting in the reporter plasmid and successfully detecting luciferase expression in each cell line (Data not shown)

The ability of virus to productively infect cells is specifically blocked by neutralizing antibodies developed against live virus. To show that JCPsV infection is dependent on properly assembled viral capsid, we used anti-JCPyV antibody to neutralize/block JCPsV infection. To simulate the infectious mode of viral entry, SVGA cells were used. JCPsV infection is significantly inhibited by antibody developed against the corresponding virus, but not by antibody developed against the closely related BK polyomavirus, or preimmune serum (Figure 2).

JCPyV uses sialic acid as a cell surface receptor to bind cells (Liu et al., 1998). JCPyV infection can be blocked by removing sialic acid with neuraminidase (Liu et al., 1998). To show that JCPsV uses the same mechanism to bind to cells as live virus, we treated SVGA cells with neuraminidase prior to infection. By removing both $\alpha(2,6)$ and $\alpha(2,3)$ – linked sialic acid, we were able to reduce JCPsV infection of SVGA cells. (Figure 3A) The interaction with sialic acid can be visualized by the virus's ability to hemagglutinate red blood cells. Dilutions correlate with the amount of virus present in a sample. We tested two dilutions of JCPsV (10^7 and 10^8 GE/mL) for their ability to hemagglutinate red blood cells and compared it to purified JCPyV and controls. We found that both dilutions of JCPsV are able to hemagglutinate, indicating that they mimic the sialic acid binding of JCPyV. (Figure

3B) The ability of the 10^8 GE dilution to hemagglutinate more than 10-fold over the 10^7 GE dilution is likely due to co-purification with pseudovirus that has packaged DNA other than the pHGluc reporter plasmid.

JCPyV uses clathrin-dependent endocytosis to enter cells, a process inhibited by chlorpromazine (Baum et al., 2003; Pho et al., 2000). Chlorpromazine was tested here for its ability to block JCPsV entry. Almost a 2-fold reduction in JCPsV infection was observed. (Figure 4A) Because SVGA cells express SV40 TAg and the reporter plasmid contains the SV40 origin of replication, plasmid replication in low numbers of infected cells can mask differences in infection. To confirm that chlorpromazine reduced infection, we also measured the number of GFP expressing cells by indirect immunofluorescence thus verifying that blocking clathrin-dependent endocytosis significantly inhibited infection. (Figure 4B) To ensure reduced infection was not due to toxicity, an MTS cell viability assay was performed. The number of cells was not reduced when treated with 5 μ M chlorpromazine (Figure 4C).

After internalization, JCPyV requires endosome acidification for productive infection (Ashok and Atwood, 2003; Eash and Atwood, 2005; Jiang et al., 2009). Ammonium chloride was used to neutralize endosome acidity (Ohkuma and Poole, 1978), while monensin and bafilomycin A1 (BafA1) were used to prevent endosome acidification. Monensin has also been shown to block BKPyV uncoating (Pietropaolo et al., 1995). BafA1 has been shown to inhibit polyomavirus endocytosis by interfering with endosomal acidification or transport (Ashok and Atwood, 2003; Liebl et al., 2006). We show that blocking this step of viral trafficking significantly inhibits JCPsV infection. (Figure 5A) To ensure reduced infection was not due to toxicity, an MTS cell viability assay was performed and we found cell number was not reduced in after drug treatment (Figure 5B).

ER trafficking has been shown to be a critical step in JCPyV infection. (Nelson et al., 2012) Thapsigargin reduces ER Ca^{2+} and prevents protein unfolding (Shmigol et al., 1995) and DTT inhibits enzymes in the ER. These two known ER inhibitors were tested for effectiveness to block JCPsV infection. Both chemicals significantly block JCPsV infection of SVGA cells, indicating that this pseudovirus mimics the ER targeting of live JC virus. (Figure 6A) An MTS cell viability assay was performed and although there was an almost 30% reduction in cells after thapsigargin treatment, this does not explain the complete obliteration of luciferase expression in treated cells. (Figure 6B)

1.4 Discussion

Polyomaviruses are difficult to study because they have very restricted tropisms at both the virus and cellular levels. (Chen and Atwood, 2002; Gee et al., 2003) We have investigated the modes of entry and trafficking for pseudovirus based on the human polyomavirus JCPyV. We first determined that JCPsV maintained the tropism of virus (Figure 1). Antibody blocking experiments, hemagglutination assays and treating cells with neuraminidase confirm that JCPsV binds sialic acid and that attachment to cells is dependent on a specific capsid-receptor interaction (Figures 2–3). We show that drugs blocking subsequent steps of viral infection can inhibit endocytosis and trafficking of pseudoviruses, demonstrating that they follow the same pathway as active/live virus (Figures 4–5). In addition, drugs that inhibit ER function inhibit pseudovirus infection, signifying that this organelle is as important for pseudovirus infection as for virus (Figure 6).

Pseudoviruses are grown in 293FT cells. These are derived from human embryonic kidney cells that have been transformed by two copies of TAg. All of the plasmids used to create pseudovirus have the SV40 origin of replication. TAg serves to drive plasmid replication and increase the output of pseudovirus. In addition to increasing pseudovirus output, this

feature is especially useful in screening human polyomaviruses, which are notorious for having low infectious titers. While pseudovirus infection can be detected in primary cells and cell lines transformed by other factors, the RLU's are routinely ten-fold higher in pseudovirus-infected TAg transformed cells (Data not shown). This allows high-throughput screening with low amounts of reagents, a combination making them ideally suited for screening large libraries of drugs or compounds.

We have shown that drugs known to block polyomavirus infection successfully inhibit polyoma-pseudovirus infection. This indicates that the pseudoviruses use the same mode of entry and trafficking as wildtype viruses. Pseudoviruses are presumably safer than viruses because they cannot replicate in cells. Pseudoviruses can be grown and purified in less than a week whereas wildtype virus growth can take over a month. Pseudoviruses are much more versatile because the reporter plasmid can be manipulated to fit different screening systems. In addition, several known JCPyV PML-type mutants are known to be defective in entry. These viruses are difficult to propagate as normal preparations depend on the virus's ability to spread in culture. PML-type mutants can easily be studied using mutated pseudoviruses. The establishment of pseudoviruses as tools for screening viral infection is a robust model and allows easier screening of drugs that inhibit viral entry and trafficking. The ability to effectively treat JCPyV-induced PML will provide protection for immunosuppressed individuals and allow safer usage of immunosuppressive therapies necessary for transplant recipients and treatment of autoimmune diseases.

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Highlights

- JCPyV causes PML in immunosuppressed patients.
- Research into antivirals is limited by difficult viral propagation.
- A JCPyV pseudovirus system mimics early events in the viral lifecycle.
- This is an efficient way to screen drugs against JCPyV entry.

Figure 1. JCPsV maintains the tropism of authentic virus

Cell lines from different species were infected with JCPsV. At 72 hours post infection luciferase expression was measured to determine whether JCPsV maintained the tropism of authentic virus. In cell lines which were negative, we verified that the reporter plasmid could be express using transfection and concluded that PsV was unable to deliver the reporter plasmid to the nucleus (Data not shown). (Ha = Hamster)

Figure 2. Entry of JCPsV is specifically blocked by neutralizing antibody against JCPyV
JCPsV was pretreated with rabbit preimmune serum, anti-BK or anti-JC for 1 hour and then used to infect SVG cells. Infection was measured 72 hours post infection (RLU). Anti-JC blocked JCPsV infection of SVGA cells ($p = 0.04$). The experiment was performed in triplicate and bars represent the standard error.

Figure 3. Removal of sialic acid inhibits JCPsV entry

3A: Cells were pretreated for 45 minutes at 37°C with PBS (pH5 with Mg²⁺ and Ca²⁺) or . 8U/100uL Type II neuraminadase (NA) in PBS (pH5 + Mg₂ and Cl₂). After infections JCPsV was removed and replaced with complete media and antibody to neutralize unbound JCPsV. Secreted luciferase was measured 72 hours post infection. Neuraminadase blocked JCPsV infection of SVG cells (p = 0.006). Error bars represent the standard error in three experiments. **3B:** JCsV behaves as JCPyV and hemagglutinates type O red blood cells. The ability of two dilutions of JCPsV genome equivalents (GE) was compared to purified wild type JCPyV and controls (33% Iodixanol in PBS, PBS, purified JCPyV or mock pseudovirus). 10⁸ JCPsV GE hemagglutinated as well as 50 uL of purified virus.

Figure 4. Disruption of clathrin-dependent endocytosis significantly reduces JCPsV infection

4A: SVGA cells were untreated or pretreated with 5 μ M chlorpromazine for 2 hours prior to infection. After infection, JCPsV was removed and replaced with drug in complete media and neutralizing antibody. Secreted luciferase was measured 72 hours post infection in three separate experiments and bars represent the standard error. Chlorpromazine treatment significantly reduced JCPsV infection by approximately half ($p = 0.001$). **4B:** To confirm this effect, we counted the number of GFP+ cells and confirmed that chlorpromazine treatment and observed a significant reduction in the number of infected cells. ($p = 4 \times 10^{-8}$) The experiment was performed in triplicate and bars represent the standard error. **4C:** To control for toxicity and cell viability, an MTS assay performed in the same well as the luciferase reading. The absorbance at 490 nm was averaged for the three experiments and bars represent the standard error.

Figure 5. JCPsV requires endosomal acidification for infection

5A: SVGA cells were pretreated with 20 mM NH₄Cl for (4 hours), 10 nM monesin, 10 nM bafilomycinA1 (BafA1) or DMSO control for 1 hr. After infection, PsV was removed and replaced with drug in complete media and neutralizing antibody. Secreted luciferase was measured 72 hours post infection. Blocking endosomal acidification significantly reduced JCPsV infection using NH₄Cl. NH₄Cl p value: 0.002; Monesin p value: 0.004; BafA1 p value: 0.01. The experiment was performed in triplicate and bars represent the standard error. **5B:** To control for toxicity and cell viability an MTS assay performed in the same well as the luciferase reading. The absorbance at 490 nm was averaged for the three experiments and bars represent the standard error.

Figure 6. Chemical inhibitors of ER function prevent JCPsV Infection

6A: SVGA cells were pretreated with DMSO, 25 nM Thapsigargin or 2.5 mM DTT for 1hr. After infection, PsV was removed and replaced with drug in complete media and neutralizing JC antibody. Secreted luciferase was measured 72 hours post infection (RLU).

6B: To control for toxicity and cell viability, an MTS assay performed in the same well as the luciferase reading. The absorbance at 490 nm was averaged for the three experiments and bars represent the standard error. Because DTT treatment interferes with the MTS assay, RLU was used to measure infectivity and cell viability was visually confirmed. Both inhibitors of ER function inhibited JCPsV infection. Thaps p value: 0.001; 5 mM DTT p value: 0.0002