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JC Polyomavirus Attachment and Entry: Potential Sites for PML Therapeutics

Colleen L. Mayberry, B.S.[1,*], **Christian D.S. Nelson, Ph.D.**[2,*], and **Melissa S. Maginnis, Ph.D.**[1,3,#]

[1]Department of Molecular and Biomedical Sciences, The University of Maine, Orono, ME, USA

[2]Biological Sciences Department, SUNY Cortland, Cortland, NY, USA

[3]Graduate School in Biomedical Sciences and Engineering, The University of Maine, Orono, ME, USA

Abstract

Purpose of review—JC polyomavirus (JCPyV) is a significant human pathogen that causes an asymptomatic infection in the kidney in the majority of the population. In immunosuppressed individuals, the virus can become reactivated and spread to the brain, causing the fatal, demyelinating disease progressive multifocal leukoencephalopathy (PML). There are currently limited treatment options for this fatal disease. Attachment to receptors and entry into host cells are the initiating events in JCPyV infection and therefore an attractive target for therapeutics to prevent or treat PML. This review provides the current understanding of JCPyV attachment and entry events and the potential therapeutics to target these areas.

Recent findings—JCPyV attachment and entry to host cells is mediated by α2,6-linked lactoseries tetrasaccharide c (LSTc) and 5-hydroxytryptamine receptors (5-HT_2Rs), respectively, and subsequent trafficking to the endoplasmic reticulum is required for infection. Recently, vaccines, monoclonal antibodies, and small molecules have shown promise as anti-viral and PML therapies.

Summary—This review summarizes our current understanding of JCPyV attachment, entry, and trafficking and the development of potential PML therapeutics that inhibit these critical steps in JCPyV infection.

Keywords

JC Polyomavirus; Progressive Multifocal Leukoencephalopathy; Natalizumab; Serotonin Receptors; VP1

[#]Please address correspondence to: Melissa Maginnis, Ph.D., Assistant Professor of Microbiology, Department of Molecular and Biomedical Sciences, University of Maine, 5735 Hitchner Hall Room 326, Orono, ME 04469-5735, (207) 581-2806, melissa.maginnis@maine.edu.

[*]These authors contributed equally to this work.

Compliance with Ethics Guidelines

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors

Introduction

JC polyomavirus (JCPyV) is the causative agent of the fatal, demyelinating disease progressive multifocal leukoencephalopathy (PML) [1]. Although PML is a rare disease, JCPyV infects the majority of the population [2, 3] and causes an asymptomatic infection in the kidney and B lymphocytes [4–7]. In severely immunosuppressed individuals, JCPyV can spread to the central nervous system (CNS) and infects astrocytes and oligodendrocytes [8–10], leading to severe demyelination and PML [11, 12].

JCPyV is a nonenveloped virus with a dsDNA genome ~5130 basepairs in size [12]. The viral capsid is comprised of three structural proteins: viral protein 1 (VP1), VP2, and VP3 [13]. VP1 is a pentameric protein present on the exterior of the capsid, and 72 VP1 pentamers interconnected through C-terminal extensions give rise to the ~40nm icosahedral capsid [14]. VP1 is the viral attachment protein that mediates binding to host cells to initiate infection [15–17]. JCPyV is internalized into host cells by endocytosis [18–20] and traffics through the endocytic compartment to the endoplasmic reticulum (ER) [21, 22], where the viral capsid is partially uncoated [23, 22]. The virus retrotranslocates from the ER to the nucleus where viral transcription and replication take place through temporally regulated gene expression [12].

The mechanisms of JCPyV attachment and entry have been the focus of research for the past several years with a particular interest in defining the cellular receptors that mediate viral attachment and entry into host cells and subsequent development of anti-viral therapies. Additionally, sequencing of viral isolates from PML patients revealed polymorphic changes that arise in the viral capsid attachment protein VP1 during viremia [24–28], representing a novel target for the development of therapeutic strategies for PML. As there are currently limited treatment options to manage JCPyV and PML, the development of an effective anti-JCPyV therapy would represent a major breakthrough. Several treatment strategies have been developed in recent years as potential methods to reduce PML disease burden including vaccines [29, 30], broadly neutralizing anti-JCPyV VP1 antibodies [31], and small molecule inhibitors [22, 32, 33]. This review summarizes the findings on JCPyV attachment, entry, and trafficking and illuminates the development of potential therapeutics targeted to block these early steps in the virus life cycle to prevent or treat PML.

Progressive Multifocal Leukoencephalopathy

JCPyV was originally isolated from the brain of an individual with PML [8, 9, 11, 34]. JCPyV infects the majority of the population [2, 3] and causes an asymptomatic infection in the kidney and can persist in B lymphocytes [4–7]. In healthy individuals, the virus remains in the kidney as a persistent infection and can be shed in the urine during times of viral reactivation [35, 36]. In individuals with severe immunosuppression, such as those with underlying immunosuppression, HIV-1 infection, or those receiving immunomodulatory therapies, JCPyV can become reactivated and spread to the CNS [27, 37] and infect astrocytes and oligodendrocytes [8, 9]. Viral-induced cell lysis of oligodendrocytes, the myelin-producing cells of the CNS, [38–40] and infection of astrocytes and glial progenitor cells (GPCs), results in severe demyelination and PML [10–12]. Additionally, JCPyV has

been detected in cortical neurons and in the granule cell neurons of individuals with PML [41–43], indicating that neuronal infection can contribute to PML pathogenesis. Demyelination in individuals with PML results in neurological dysfunction and patients develop symptoms including cognitive disorders, motor weakness, language disturbances, and visual defects [44]. PML is a rapid, progressive disease that usually results in fatality within months to 1 year of symptom onset [45]. If the underlying immunosuppression is treated, such as with highly-active antiretroviral therapy (HAART) for HIV-1 infection or by removing the immunosuppressive therapy to restore immune function, the life span of the patient can be prolonged [45, 46]. However, individuals treated for immune reconstitution are at risk for development of immune reconstitution inflammatory syndrome (IRIS), which can lead to neurological worsening, and the patients are oftentimes severely debilitated [46].

HIV-1 infection accounts for 80% of PML cases and ~5% of HIV-1+ individuals develop PML, which is considered an AIDS-defining illness [47]. The majority of non-HIV-PML cases are due to the use of immunomodulatory therapies for immune-mediated diseases [48–50]. In the past decade, the number of PML cases has risen in patients receiving an FDA-approved drug, natalizumab (Tysabri®), for treatment of multiple sclerosis (MS) [49]. Natalizumab is a humanized monoclonal antibody (mAb) specific for very late antigen-4 (VLA-4) (α4β1 integrin) [50] that blocks autoreactive VLA-4^+ T- and B-lymphocyte trafficking to the brain [51] to prevent the T cells from attacking the myelin in the CNS, the key feature of the disease etiology of MS [49, 52]. As of March 6, 2017, there have been 711 natalizumab-associated cases of PML [53], as well as PML cases in individuals receiving other humanized mAb treatments including rituximab, efalizumab, and infliximab [49, 50, 54–56]. Newly developed drugs including fingolimod [57], dimethyl fumarate drugs, and other fumaric acid ester-containing drugs have also caused cases of PML [58–61]. While some cases of PML have occurred in individuals for whom these drugs were prescribed for immune-mediated diseases including Crohns disease or rheumatic disease [62, 63], the majority of PML cases occurred when these drugs were prescribed for MS [49]. It is likely that the physiological aspects of this disease, damage to the CNS by autoreactive T cells [52], in combination with a mAb therapy that prevents immune surveillance in the CNS [49, 64], may present an environment that promotes JCPyV neuroinvasion. While these drugs have all been associated with the development of PML, the relative benefit to the patient combined with risk stratification of PML development is taken into consideration [65], and the prescribing information for drugs like Tysabri® contains a black box warning [53]. In fact, the drug ocrelizumab that was recently FDA-approved for both relapsing and primary progressive MS, targeting $CD20^+$ B cells, has not resulted in any cases of PML during the Phase III clinical trials [66, 67], yet also comes with a warning about the potential risk of PML [68]. The rise in the development of immune-mediated therapies and the related increase in immunomodulatory therapy-associated PML cases together with HIV-1-related PML cases highlight a critical need for the development of improved PML treatments.

JC Polyomavirus Attachment to Host Cells

The initial attachment of viruses to host cells is mediated by cellular receptors, and JCPyV binds specifically to sialic acid containing receptors, a common feature shared by polyomaviruses and other viruses [69, 70]. A number of sialic acid containing receptors have

been studied for their role in supporting JCPyV infection including α2,3- and α2,6-containing sialic acid receptors [16, 17, 27, 71–74]. A structural-functional approach demonstrated that JCPyV utilizes a specific α2,6-containing sialic acid receptor known as lactoseries tetrasaccharide c (LSTc), which was identified using a glycan array screen with purified VP1 pentamers of the JCPyV-1a strain Mad-1 [17] (Fig. 1). JCPyV VP1 did not bind to any other glycans included on the array, including any of the sialic acid-containing ganglioside receptors [17], which are utilized by all other known polyomaviruses [17, 75–78] and have been suggested as receptors for JCPyV [27, 72]. Interestingly, using other techniques JCPyV has been demonstrated to bind to ganglioside receptors, albeit with significantly reduced affinity, and gangliosides do not support infection [74]. The X-ray crystal structure of JCPyV VP1 in complex with LSTc demonstrated specific residues required for VP1 binding to LSTc (L54, N123, S266, and S268, also referred to L55, N124, S267, and S269 in conventions counting the Met start codon) [17]. Mutation of the residues critical for sialic acid binding in the context of an infectious viral clone and purified VP1 pentamers revealed that these residues are essential for growth and binding in glial cells [17]. Interestingly, mutations in the exact residues that are critical for LSTc attachment and infection of cells *in vitro* are found to arise in individuals with PML [25–28].

PML-associated VP1 Mutations

Samples of the cerebral spinal fluid (CSF) from individuals with PML reveal that ~90% of the viral isolates have at least one point mutation or combinations of mutations in VP1 in residues L54, N123, S266, or S268 [25–28]. These VP1 mutations are not usually found in isolates from the urine, indicating that perhaps VP1-associated mutations are linked to viral spread to the CNS or favor PML onset [25–28]. Interestingly, PML-associated mutations in VP1 results in abolished binding to LSTc as determined by X-ray crystallography [79], and pseudoviruses engineered with PML-associated mutations were not capable of transducing a range of brain cell types or hemagglutinating human red blood cells indicating a loss of sialic acid binding [79]. These *in vitro* studies indicate that virions with PML-associated mutations would be non-infectious in the host [79]. However, these mutations were generated in the background of the Mad-1 laboratory prototype strain, which is of the viral genotype 1a, and, there are seven genotypes used to classify JCPyV strains based on differences in the VP1 amino acid sequences [80]. Introduction of PML-associated mutations into the background of the JCPyV-2a strain have resulted in viruses that are infectious *in vitro* in oligodendrocytes, astrocytes, and glial progenitor cells (GPCs) and *in vivo* in a chimeric mouse model with explanted GPCs [10]. Interestingly, viruses collected from mice after infection with wild type JCPyV-2a demonstrated PML-associated mutations D66G and S123C, which are within the sialic acid binding pocket and arise in human patients [17]. Further, JCPyV-2a pseudoviruses with PML-associated mutations such as S266F can transduce some cancer cell lines [29]. Thus, the specific genotypic background of PML strains seems to be an important factor for PML-associated mutations in PML progression *in vivo* and for growth *in vitro*. Further, alternate routes of receptor-independent viral entry and additional cell types need to be tested to determine the infectious nature of these mutants. It is possible that JCPyV binding to and infection of cells in the brain including, astrocytes, oligodendrocytes, and neurons, is sialic-acid-independent in an *in vivo*

infection. Haley et al. demonstrated that astrocytes and oligodendrocytes from human brain tissues were negative for LSTc [81]. Therefore, viruses isolated from PML patients with mutations in VP1 within the sialic acid binding sites could possibly lead to neuroinvasion via a sialic acid-independent manner through interactions with an alternate receptor or through a receptor-independent invasion mechanism that has been demonstrated for other viruses [82, 83]. Further research is necessary to define whether viruses with mutations in VP1 are the infectious form of the virus or whether they arise during CNS invasion and contribute to PML pathogenesis through an alternative mechanism.

PML Treatments Targeted to VP1

The incidence of PML-associated mutations in PML patients and in animal model systems indicate a correlation for PML-associated mutations and PML development. These findings further demonstrate that VP1 is a key target for antivirals and activation of humoral and cell-mediated immunity [31]. Recent studies have focused on vaccine or mAb therapies in combination with treatments to boost VP1-specific immunity (Table 1). For instance, two patients were treated under “compassionate use” with a vaccine consisting of JCPyV VP1 protein in combination with cytokine interleukin 7 (IL-7) treatment and a toll-like receptor (TLR) 7/9 agonist as an adjuvant [30]. This treatment led to JCPyV clearance with an undetectable viral load and patient recovery [30]. Interestingly, sera isolated from PML patients can effectively neutralize wild-type JCPyV, yet does not neutralize virus with PML-associated mutations, while sera from healthy patients can neutralize wild-type and PML variants [29, 31]. These findings suggest that patients who develop PML have antibody “recognition holes” during immunosuppression, and their antibodies cannot neutralize variants with PML-associated mutations such as L55F, S266F, and S269F [29, 31]. However, during immune reconstitution, the antibody titers in the CSF rise and the antibodies can recognize the PML-associated variants, which may be essential for neutralization and elimination of the virus [29, 31]. Thus, therapies to effectively neutralize JCPyV with PML-associated mutations and subsequently boost immunity are being developed [29, 31]. Vaccines generated from wild-type JCPyV-2a virus-like particles (VLPs) and administered to mice initially result in “recognition holes” that prevent neutralization of PML variants, yet these holes are closed when mice are administered a booster [29]. The “compassionate use” of a VLP vaccine in combination with IL-7 in a patient resulted in an increase in neutralizing antibody titer with antibody production specific for the PML-associated mutant found in the patient’s CSF and clinical improvements including reduced viremia and halted progression of PML lesions [29]. Furthermore, antibodies derived from memory B cells from healthy patients and patients with PML-IRIS were expanded through molecular cloning of antibodies capable of neutralizing JCPyV infection [31]. Antibodies from a natalizumab-PML-IRIS patient were found to have high neutralizing activity, high affinity for VP1, and recognition capacity for PML variants, providing a novel mAb-based strategy that could potentially neutralize JCPyV infection in PML patients [31]. This treatment could be particularly effective in individuals who develop PML-associated mutations during viremia that cannot be effectively neutralized by the compromised immune system and succumb to PML. Taken together, active or passive immunity with a VLP vaccine, VP1, or VP1-specific

monoclonal antibodies in combination with treatments that boost host immunity represents a promising new target for PML treatment.

Serotonin Receptors in JCPyV Entry

JCPyV enters host cells by clathrin-mediated endocytosis as JCPyV infection is significantly reduced by chlorpromazine, an inhibitor of clathrin-mediated endocytosis [18, 20]. Further, expression of dominant negative mutants of (epidermal growth factor receptor kinase substrate clone 15) eps15, a protein important for clathrin-mediated endocytic events, reduces infection [19]. Chlorpromazine is also a 5-hydroxytryptamine (5-HT) (serotonin) receptor antagonist that acts as an inhibitor of 5-HT2A serotonin receptor (5-HT_{2A}R) [84], which led to the hypothesis that JCPyV utilizes 5-HT_{2A}R as a proteinaceous receptor. Subsequently, the Atwood laboratory identified that 5-HT_{2A}R is required for JCPyV infection [85], based on the findings that infection of glial cells was sensitive to the use of 5-HT_2R-blocking antibodies specific to subtypes 2A and 2C, 5-HT_{2A}R expression rendered HeLa cells permissive for infection, and JCPyV colocalized with 5-HT_{2A}R-GFP at time points consistent with viral entry [85]. Furthermore, 5-HT_{2A}R antagonists including ritanserin, ketanserin, mianserin, and mirtazapine reduced JCPyV infection in human glial cells [86]. Collectively, these data indicate that 5-HT_2Rs are important for JCPyV infection and likely promote viral entry, most likely through clathrin-mediated endocytosis.

The 5-HT_2Rs are seven-transmembrane-spanning G-protein coupled receptors widely expressed in the CNS and are commonly associated with physiologic and mood disorders [87]. Interestingly, 5-HT_2Rs are expressed on a variety of cells in the CNS, including astrocytes and oligodendrocytes, and in the kidney, including the distal tubules and collecting ducts, all of which are sites of JCPyV infection [81, 88, 89]. Furthermore, 5-HT_2Rs are expressed on neurons, and subtype 2A is found abundantly in the cerebral cortex [90] where JCPyV has been identified in sites of significant demyelination in individuals with PML [41, 43]. Interestingly, JCPyV is not able to infect microglia, cells that express 5-HT_2Rs but lack expression of the JCPyV attachment factor LSTc, further indicating that 5-HT_2Rs are not the sole requirement for viral infection [81].

Given that treatment of cells with inhibitors and antibodies specific for serotonin subtypes 5-HT_{2A} and 5-HT_{2C} have shown diminished JCPyV infection, it was speculated that multiple 5-HTR subtypes may be capable of conferring infection or multiple subtypes may play functionally redundant roles in infection. Expression of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors increased the susceptibility of poorly permissive HEK293A cells, which express low levels of 5-HTRs, to JCPyV infection while other 5-HTR subtypes did not [20]. The presence of 5-HT_2Rs in HEK293A cells did not impact viral attachment to the cell surface, but specifically enhanced viral entry that was blocked by chlorpromazine treatment [20]. These data demonstrate that the 5-HT_2Rs subtypes are required for JCPyV entry [20], yet the direct entry mechanism remains unclear as chlorpromazine blocks clathrin-dependent endocytosis and is also a 5-HT_{2A}R antagonist [84] (Fig. 1). Interestingly, JCPyV was demonstrated to infect human brain microvascular endothelial cells (HBMECs), which lack 5-HT_{2A}Rs, indicating that infection could occur in the absence of 5-HT_{2A}R [91]. However, these findings predate evidence that JCPyV can also use 5-HTR subtypes 2B and 2C [20].

Furthermore, HBMECs are primary endothelial cells in contrast to the aforementioned HEKs, which are transformed epithelial cells. Therefore, entry strategies could vary in a tissue-specific manner or based on cellular transformation, and thus requires further investigation.

Given the evidence that JCPyV entry and infection are dependent on 5-HT_2Rs and the abundance of on-market therapies that target the 5-HT_2Rs, such as selective serotonin reuptake inhibitors (SSRIs) for use in depression treatment [87], clinicians have prescribed 5-HT_2R antagonists, including mirtazapine, in an off-label treatment for PML. Mirtazapine is a serotonergic antagonist that selectively inhibits 5-HT_2 and 5-HT_3 receptors [92]. Interestingly, case reports have indicated varying degrees of efficacy following mirtazapine treatments in PML patients. Several studies show that treatment with mirtazapine dramatically improves prognosis for individuals diagnosed with PML, including decreased neurological deterioration and undetectable viral loads [93, 94]. However, other reports have described instances where treatment with mirtazapine had little beneficiary effect, as patients rapidly deteriorated regardless of 5-HT_2R antagonist treatment [95, 96]. As mirtazapine blocks 5-HT_2Rs it is tempting to speculate that treatment likely prevents JCPyV spread to other cells rather than treating established infection as MRI scans have remained unchanged in some cases [93, 95, 96]. Moreover, other reports indicate that mirtazapine, in combination with mefloquine, a treatment marketed for malaria, has been effective in treating some individuals with PML [97]. Treatment of cells with mefloquine decreased JCPyV infection by blocking viral replication, indicating that the combination treatment may be effective in treating PML [98]. While these reports are promising, conflicting evidence indicates that JCPyV-mediated infection by 5-HT_2Rs requires further investigation as well as continued exploration of 5-HT_2R antagonists as viable treatments for PML.

JCPyV Trafficking

Following internalization into host cells through a mechanism involving 5-HT_2Rs, JCPyV enters the endocytic pathway and is observed in Rab5-positive early endosomes by 15 minutes post infection [21]. Rab proteins are small GTPases that play critical roles in controlling endocytic trafficking, sorting, motility, and fusion [99]. Localization of JCPyV to early endosomes appears to be a critical step, as overexpression of dominant-negative forms of Rab5 restrict JCPyV infection [21]. Localization to Rab5-positive early endosomes is in contrast to other polyomaviruses (Pys) such as Simian Virus 40 (SV40) and mouse polyomavirus (mPyV), which have been shown to accumulate within Rab7-positive maturing or late endosomes [100, 101]. Other Pys have been shown to initially interact with gangliosides on the surface of the cell, and the differences in entry pathways between JCPyV and other Pys may be due to JCPyV utilizing a secondary proteinaceous receptor for entry following sialic acid receptor interactions, differing from other Pys studied [20, 102–105]. JCPyV has also been observed in cholesterol rich and caveolin-1 (Cav-1) positive endosomes, and shRNA-mediated reduction of Cav-1 or sequestration of cholesterol with the drug Methyl-β-cyclodextrin reduces infection [21]. Although the entry pathway of JCPyV appears to be distinct from other Pys, they all appear to require caveolin-1+ vesicles for trafficking to the ER, implying overlapping events in endosomal and caveolin-mediated trafficking strategies [104–107]. As JCPyV has been shown to require tyrosine kinase

activity and actin rearrangement, this suggests that microfilaments may be important for infection and trafficking within endosomal compartments following entry [19, 108].

Retrograde Transport of JCPyV

The actual site of egress from the endosomal-lysosomal system remains enigmatic for JCPyV. JCPyV has not been observed in Rab7-positive maturing or late endosomes, and overexpression of dominant-negative forms of Rab7 does not inhibit infectivity [19]. These observations suggest that JCPyV leaves the endosomal-lysosomal system from early endosomes (Fig. 1) [21]. The early endosome is a complex organelle that contains microdomains involved in endosomal maturation (vacuolar domain) and sorting (tubular endosomal network) (reviewed in [109]). Transport of cargo through the tubular endosomal network involves a number of proteins or protein complexes including clathrin, retromer, and the WASH complex [110, 111]. It is unclear whether JCPyV utilizes these host factors to initiate transport from the early endosome and future studies are needed to definitively identify the host factors responsible for JCPyV egress from the endosomal-lysosomal system.

Retrograde transport from endosomes results in movement of cargo to the trans-Golgi network [112]. However, colocalization between JCPyV virions (or other Pys) with the Golgi apparatus has not been described [23, 100, 101, 104]. It is currently unknown whether JCPyV undergoes direct endosome to ER transport, or transiently localizes to the Golgi prior to arrival in the ER. JCPyV accumulates in the host cell ER starting around 4–6 hours post infection [21, 23]. While the kinetics of JCPyV arrival to the ER is on par with other Pys, it is slow compared to other cargo targeting the ER [101, 113, 114]. Studies with other Pys have demonstrated that virus-receptor interactions are important for directing virions to the ER [100, 101, 115], and future quantitative studies using both virus and receptor labeling strategies may help to elucidate the retrograde transport pathway used by JCPyV.

Small Molecule-Mediated Inhibition of JCPyV Retrograde Transport

Presently, it is thought that JCPyV traffics to the ER where the virus interacts with components of the host cell quality control machinery, such as PDI and ERP57, to undergo partial uncoating of the viral capsid [23]. It is speculated that this partially uncoated capsid then undergoes retrotranslocation from the lumen of the ER into the cytosol using components of the endoplasmic reticulum associated degradation (ERAD) pathway [23]. Uncoating is a key step in the virus life cycle that results in release of the viral genome into the host nucleus, and therefore presents an attractive target for therapeutic development. Retro-2, a small molecule inhibitor of retrograde transport, was found to inhibit infection of JCPyV and other Pys [22]. Retro-2 acts on an unidentified host factor to inhibit retrograde transport from endosomes (Fig. 1) [116]. In addition, Retro-2 has also been shown to inhibit JCPyV pseudovirus transduction in several kidney and glial cell lines, suggesting that Retro-2 targets a conserved transport machinery. Small molecule inhibitors that target host cell factors run the risk of having deleterious side effects, but also reduce the likelihood that viruses will be able to generate escape mutations allowing infection [117–119]. Importantly, follow-up studies have produced chemical analogs of Retro-2 that inhibit viral infectivity at

over 5-fold lower concentrations than Retro-2 [32]. These results suggest that continued development of Retro-2 or chemical analogs may eventually lead to drugs that provide effective treatment options for individuals suffering from JCPyV infection and PML. Future drug development studies will likely also need to examine the ability of Retro-2 or related compounds to cross the blood brain barrier in order to neutralize viral replication in the CNS.

Conclusions

JCPyV infection occurs in the majority of the population [2, 3] yet only results in PML in severely immunosuppressed individuals such as those with HIV-1 infection [47] or those under immunosuppressive therapies [49]. Viral attachment to host cells in culture is dependent on α2,6-sialic acid on LSTc via interactions with specific residues on VP1 [17]. Mutations arise in the sialic acid binding pocket of VP1, and these sites cannot be neutralized by antibodies isolated from individuals that are immunosuppressed [29, 31]. Individuals that recover from PML develop antibodies to PML-associated mutations that can lead to neutralization and recovery in combination with immune system activators [31]. VP1-based vaccine combination treatment to boost anti-VP1 immunity have led to clinical improvements in PML patients [29, 30], and VP1-specific mAbs represent a new potential treatment option [31]. Following attachment, JCPyV enters cells by 5-HT_2Rs [20, 85], and although antagonists to 5-HT_2Rs have shown some clinical promise [93, 94], there are other instances in which no clinical improvement has been observed [95, 96]. Novel small molecule inhibitor Retro-2 that blocks retrograde transport of the virus from the ER at lower concentrations shows promise *in vitro* [22, 32]. Taken together, viral attachment, entry, and trafficking represent novel targets for anti-viral therapeutics, presenting an opportunity for the continued development of improved PML treatments.

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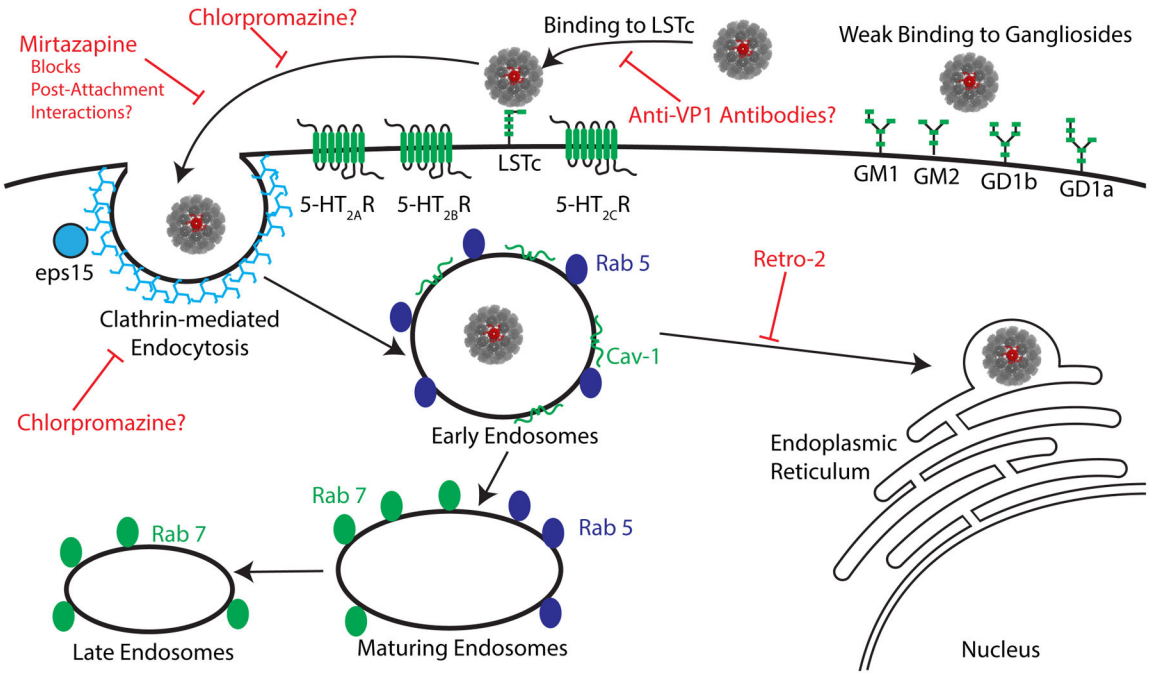

Fig. 1. Attachment and entry of JCPyV into host cells and potential targets for PML treatment

JCPyV binds to α2,6 sialic acid-containing receptor lactoseries tetrasaccharide c (LSTc) through interactions with the viral capsid protein VP1 (pentamer in red) to initiate infection of susceptible cells. JCPyV binds with weak affinity to sialic acid-containing gangliosides, however, this interaction does not appear to lead to productive infection. Following interactions with LSTc, JCPyV enters cells through clathrin-mediated endocytosis in an EPS15-dependent manner that is sensitive to chlorpromazine treatment. The serotonin 2 subfamily (5-$HT_{2A, 2B, 2C}$) of receptors play an important role in viral internalization, yet are not thought to contribute to virus binding. Mirtazapine and chlorpromazine interfere with viral infection, possibly by disrupting JCPyV interactions with serotonin receptors. Following endocytosis, JCPyV likely accumulates in Rab5-positive early endosomes. JCPyV also localizes with Cav-1 positive vesicles, but it is currently unclear whether these are also early endosomes. The virus then undergoes retrograde transport to the ER, a step that is sensitive to Retro-2 treatment.

Table 1

Potential treatments highlighted in this article that have been shown to reduce JCPyV infection and/or PML progression.

Potential PML Treatment	FDA Licensed	Mechanism of Action	Tested in Clinical Setting
Mefloquine	Yes	Viral Replication Inhibitor	Yes
Mirtazapine	Yes	Serotonin Receptor Antagonist – Possible Receptor Competition	Yes
Retro-2	No	Intracellular Transport Inhibitor	No
VP1 Subunit Vaccine	No	Active Immunization	Yes
VLP Vaccine	No	Active Immunization	Yes
Anti-VP1 mAb	No	Passive Immunization	Yes
LSTc competitive inhibitors	No	Receptor Competition	No