A Retrograde Trafficking Inhibitor of Ricin and Shiga-Like Toxins Inhibits Infection of Cells by Human and Monkey Polyomaviruses

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ABSTRACT Polyomaviruses are ubiquitous pathogens that cause severe disease in immunocompromised individuals. JC polyomavirus (JCPyV) is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), whereas BK polyomavirus (BKPyV) causes polyomavirus-induced nephropathy and hemorrhagic cystitis. Vaccines or antiviral therapies targeting these viruses do not exist, and treatments focus on reducing the underlying causes of immunosuppression. We demonstrate that retro-2^{ cycl}, an inhibitor of ricin and Shiga-like toxins (SLTs), inhibits infection by JCPyV, BKPyV, and simian virus 40. Retro-2^{ cycl} inhibits retrograde transport of polyomaviruses to the endoplasmic reticulum, a step necessary for productive infection. Retro-2^{ cycl} likely inhibits polyomaviruses in a way similar to its ricin and SLT inhibition, suggesting an overlap in the cellular host factors used by bacterial toxins and polyomaviruses. This work establishes retro-2^{ cycl} as a potential antiviral therapy that broadly inhibits polyomaviruses and possibly other pathogens that use retrograde trafficking.

IMPORTANCE The human polyomaviruses JC polyomavirus (JCPyV) and BK polyomavirus (BKPyV) cause rare but severe diseases in individuals with reduced immune function. During immunosuppression, JCPyV disseminates from the kidney to the central nervous system and destroys oligodendrocytes, resulting in the fatal disease progressive multifocal leukoencephalopathy. Kidney transplant recipients are at increased risk of BKPyV-induced nephropathy, which results in kidney necrosis and loss of the transplanted organ. There are currently no effective therapies for JCPyV and BKPyV. We show that a small molecule named retro-2^{ cycl} protects cells from infection with JCPyV and BKPyV by inhibiting intracellular viral transport. Retro-2^{ cycl} treatment reduces viral spreading in already established infections and may therefore be able to control infection in affected patients. Further optimization of retro-2^{ cycl} may result in the development of an effective antiviral therapy directed toward pathogens that use retrograde trafficking to infect their hosts.
host cellular machinery used to promote ER targeting of virions remains unclear.

In this study, we demonstrate that the small molecule 2-[(5-methyl-2-thienyl)methylene]amino]-N-phenylbenzamide (retro-2 cycl) potently inhibits the infection of tissue culture cells by JCPyV, BKPyV, and the closely related primate virus simian virus 40 (SV40). Retro-2 cycl was previously identified by high-throughput screening for small molecules that inhibit the intoxication of host cells by ricin, Shiga-like toxins (SLTs), and the cholera toxin B subunit (CTxB) (20). Retro-2 cycl inhibits retrograde trafficking from endosomes to the Golgi apparatus, thus preventing the intoxication of host cells. Rather than binding to the toxins, retro-2 cycl interferes with retrograde trafficking of cargo by interaction with an unidentified cellular host factor. We demonstrate that retro-2 cycl inhibits polyomavirus infection by inhibiting ER transport, suggesting that polyomaviruses, ricin, SLTs, and CTxB share a dependency on similar retro-2 cycl sensitive host factors for successful intracellular transport. Recently, we found that retro-2 cycl also inhibits the infection of cells by human papillomaviruses (21). Further optimization of this compound may result in the development of effective antiviral compounds that inhibit the infection of cells by viruses requiring ER transport for infection.

RESULTS

Retro-2 cycl inhibits polyomavirus infection in a dose-dependent manner. To determine whether retro-2 cycl inhibits infection by polyomaviruses, we pretreated permissive cells with retro-2 cycl and infected them with JCPyV, BKPyV, or SV40 (20). Retro-2 cycl treatment resulted in a dose-dependent decrease in infected cells compared to a vehicle control, with calculated 50% effective concentrations of 28.4, 61.2, and 58.6 μM for JCPyV, BKPyV, and SV40, respectively (Fig. 1A). As a control, we pretreated Vero cells with retro-2 cycl and inoculated them with a green fluorescent protein (GFP)-expressing adenovirus (Ad5-GFP), which is known not to require retrograde trafficking (22). Retro-2 cycl treatment did not inhibit adenovirus-mediated transduction, suggesting that the effect of retro-2 cycl on infection is specific to virions that undergo retrograde trafficking (Fig. 1B). We additionally infected a panel of cell lines with JCPyV pseudovirus expressing a luciferase reporter gene. Retro-2 cycl exhibited low cellular toxicity at protective levels (see Fig. S2).
Retro-2<sup> cycl</sup> is able to reduce viral spreading in established tissue culture infections. Since most individuals are persistently infected with JCPyV or BKPyV prior to immunosuppression, we asked whether retro-2<sup> cycl</sup> could prevent viral spreading in established tissue culture infections. Cells were infected at a low multiplicity of infectivity (MOI) of 0.01. Following one round of productive infection, 100 μM retro-2<sup> cycl</sup> was added to these cells and maintained during the course of the experiment. Treatment of cells resulted in a significant reduction of infected cells compared to the vehicle control, and this effect was most striking at 12 days postinfection, where retro-2<sup> cycl</sup> diminished the spreading of SV40 (84% inhibition), BKPyV (89%), and JCPyV (90.5%) (Fig. 1C). To examine whether the treatment of these cultures with retro-2<sup> cycl</sup> inhibited virion production, supernatants from each time point were used to infect naive cells that were not retro-2<sup> cycl</sup> treated. Cultures that were previously treated with retro-2<sup> cycl</sup> produced significantly less infectious virions (Fig. 1D), demonstrating that retro-2<sup> cycl</sup> decreases the cell-to-cell spreading of polyomaviruses in previously infected cultures.

The bioactive compound is a DHQ derivative of retro-2. After showing that commercially purchased retro-2 inhibits polyomavirus infection, we chemically synthesized the compound by a previously reported method in order to facilitate subsequent investigations (23). Condensation of 2-aminobenzanilide with 4-methyl-2-thiophencarboxaldehyde in the last step of synthesis yielded a mixture of two products, both having the expected molecular weight of retro-2 (see Fig. S3A in the supplemental material). The two compounds were separated and independently characterized. One product was retro-2, as indicated by a characteristic singlet at 11.0 ppm for the imine proton, as determined by nuclear magnetic resonance analysis (data not shown). The second product was revealed to be a dihydroquinazolinone (DHQ) by X-ray diffraction crystallography and is termed retro-2<sup> red</sup> (Fig. 2A). The spectroscopic data for the compound purchased from ChemBridge were identical to those for the DHQ derivative and not to those of the structure reported for retro-2 (data not shown). To test the inhibitory effects of both compounds on JCPyV infection, SVG-A cells were incubated with 100 μM retro-2 or retro-2<sup> red</sup> and challenged with JCPyV. Surprisingly, both retro-2 and retro-2<sup> cycl</sup> inhibited polyomavirus infection with similar efficacies (Fig. 1B).

Imine species similar in structure to retro-2 are commonly invoked as mechanistic intermediates in the formation of DHQ (24–28), which suggests that the two chemical species could interconvert in the infectivity assay, thus accounting for their similar biological activities. Accordingly, we found that treatment of retro-2 with scandium(III) trifluoromethanesulfonate in methanol resulted in rapid conversion to the DHQ (data not shown). In aqueous medium, retro-2 most likely cyclizes into a DHQ as well. During the preparation of this report, another group reported that retro-2 slowly cyclizes in methanol and named this compound “Retro-2<sup> cycl</sup>” (29).

It was still unclear whether the biologically active compound was an imine or a DHQ. Treatment of retro-2<sup> cycl</sup> with sodium cyanoborohydride in methanol slowly produced a reduced species (retro-2<sup> meta</sup>), which indicated that cyclization is reversible and that retro-2 and retro-2<sup> cycl</sup> exist in equilibrium (see Fig. S3B in the supplemental material). However, despite their structural similarities, retro-2<sup> meta</sup> is significantly less active than retro-2 (Fig. 2B). We also prepared a retro-2 regiosomer wherein the carboxamide and imine moieties are meta substituted (retro-2<sup> meta</sup>), therefore precluding cyclization (see Fig. S3C). This compound was also significantly less active and served as a useful negative control in subsequent experiments. Together, the lack of biological activity intrinsic to retro-2<sup> red</sup>, as well as retro-2<sup> meta</sup>, leads us to the conclusion that the chemical species responsible for inhibition of polyomavirus infection is, in fact, the DHQ, retro-2<sup> cycl</sup>.

Retro-2<sup> cycl</sup> inhibits polyomavirus infectivity at early time points during infection. We hypothesized that retro-2<sup> cycl</sup> inhibited retrograde trafficking of polyomavirus to the ER. SVG-A or Vero cells were synchronously infected with JCPyV, BKPyV, or SV40, and retro-2<sup> cycl</sup> was added to cells at the indicated time points. The results show that the addition of retro-2<sup> cycl</sup> at time points up to 4 h postinfection (hpi) significantly reduces infectivity, with a progressive loss of its inhibitory effect from 6 to 18 hpi (Fig. 3A). These kinetics are consistent with previous reports showing that polyomaviruses colocalize with ER markers at 6 to 16 hpi, demonstrating that the protective effect of retro-2<sup> cycl</sup> is lost
Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2cycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2cycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2ycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2ycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2ycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2ycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2ycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2cycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2cycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2cycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2cycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2cycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2cycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.

Retro-2cycl inhibits polyomavirus infectivity at early time points during infection. (A) Cells were chilled prior to incubation with JCPyV, BKPyV, or SV40 to synchronize infections, and retro-2ycl (0.1 mM) was added at the indicated time points. After 72 h, infected cells were scored and normalized to the vehicle control. The data represent the mean of three replicates, and error bars indicate the standard deviation. (B) Effect of retro-2cycl on virus binding to cells. Cells were detached and pretreated with the vehicle control or retro-2cycl for 30 min at 37°C. Cells were then inoculated with Alexa Fluor 633-labeled JCPyV, BKPyV, SV40, or CTxB for 1 h on ice. Samples were then washed and read by flow cytometry. CTB, CTxB.
A critical step in the infectious entry of polyomaviruses is ER transport, and we show that retro-2\textsuperscript{cycl} significantly reduces the transport of virions to the ER (32, 33). Since virions cannot interact with ER-resident chaperones in retro-2\textsuperscript{cycl}-treated cells, necessary uncoating steps are inhibited, as evidenced by a lack of exposure of the minor capsid protein VP2 in retro-2\textsuperscript{cycl}-treated samples. All of the polyomaviruses studied to date undergo ER transport, and in recent years, nine new human polyomaviruses have been discovered (34). Several of these newly discovered viruses are associated with human diseases, including Merkel cell polyomavirus, which is the causative agent of the fatal cancer Merkel cell carcinoma (35). Since retro-2\textsuperscript{cycl} is protective against JCPyV, BKPyV, and SV40, it is likely that this compound will inhibit the replication of these new polyomaviruses and will be a useful tool in verifying whether these new polyomaviruses target

**FIG 4** Retro-2\textsuperscript{cycl} inhibits polyomavirus ER trafficking. (A) Colocalization was assessed with a PLA. Error bars denote the standard deviation. (B) Cells were pretreated with the indicated drug (500 ng/ml BFA or 0.1 mM retro-2\textsuperscript{cycl}) for 0.5 h prior to inoculation with JCPyV, BKPyV, or SV40 at an MOI of 100. Cells were incubated for 8 h with the indicated drugs, fixed, and permeabilized. Cells were then stained with a mouse monoclonal antibody to PDI and a rabbit polyclonal antibody to VP1 prior to detection by PLA. Fluorescent foci indicate areas of colocalization. BKV, BKPyV.

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**FIG 5** Retro-2\textsuperscript{cycl} inhibits VP2 exposure of polyomaviruses. (A) VP2 is exposed at late time points during infection. Cells were pretreated with the indicated drugs and then inoculated with JCPyV, BKPyV, or SV40 at an MOI of 10 for 10 h before fixation and staining for VP2. VP2 puncta are green, and nuclei are blue. Scale bars, 10 μm. (B) VP2 is exposed in the ER. Cells were incubated with SV40 for 10 h, fixed, and then stained for VP1 (green), VP2 (red), and PDI (purple), and the nuclei were stained with BOBO-3 (blue). On the right, enlargements of the boxed area of the fluorescence micrograph show individual antibody staining. (C) Quantitation of panel A. Cells from triplicate samples were scored for the presence of VP2. Error bars show the standard deviations.
the ER for productive infection. We also found that pretreatment of cells with retro-2\textsuperscript{cycl} had no effect on the binding of JCPyV or CTxB to cells but did slightly reduce the binding of BKPyV and SV40. This is unlikely to be due to reductions in cell surface receptor expression, as the SV40 receptor, GM1, was not reduced, as evidenced by the binding of CTxB. This was also not due to direct binding of retro-2\textsuperscript{cycl} to the virions, as incubation of labeled viruses with retro-2\textsuperscript{cycl} did not reduce binding. Finally, treatment of the cells did not prevent any of these viruses from entering the cell. The major effect is at the level of intracellular trafficking to the ER.

BFA is another small molecule that has been reported to inhibit the ER accumulation of polyomaviruses (30, 31, 36–38). However, BFA is highly cytotoxic to cells, making this molecule less appealing for the development of antiviral or antitoxin therapies (39). Additionally, BFA treatment rapidly alters the morphology of the Golgi apparatus, inhibits endosomal maturation, and inhibits protein secretion, demonstrating that this compound elicits numerous effects besides inhibiting retrograde trafficking (40–43). Conversely, retro-2\textsuperscript{cycl} does not alter cell compartment morphology and is well tolerated when administered to mice (20). Thus, retro-2\textsuperscript{cycl} is likely the first small-molecule inhibitor of polyomavirus infectivity that shows promise as a potential antiviral therapy.

We also show that the biologically active chemical species of retro-2 is a DHQ derivative of retro-2 and not an imine, as was originally reported (20). While we were completing these studies, another group confirmed that retro-2 is converted to a DHQ (29). The correct structure of the retrograde transport inhibitor now established, we can consider the medicinal chemistry optimization of retro-2\textsuperscript{cycl} as a potential drug lead.

The inhibitory effect of retro-2\textsuperscript{cycl} is strikingly similar to the effect seen on ricin toxin and Shiga-like toxins, where retro-2\textsuperscript{cycl} treatment prevents endosome-to-Golgi apparatus trafficking and, as a consequence, also inhibits ER trafficking (20). This suggests that there may be an overlap of the cellular proteins used by toxins and polyomaviruses to effect ER transport. However, there are likely significant differences in the kinetics or pathways used by polyomaviruses and bacterial toxins to target the ER, since ricin and Shiga-like toxins rapidly traffic to the Golgi apparatus (44), an association that has yet to be identified for any polyomavirus. This suggests that the cellular host factors targeted by retro-2\textsuperscript{cycl} may be involved in multiple retrograde trafficking pathways, that only a small proportion of virions traffic to the Golgi compartment, or that polyomaviruses may rapidly traffic through the Golgi complex prior to ER accumulation. SLTs, CTxB, and some polyomaviruses bind to glycolipids and may therefore provide a rationale for how this compound inhibits trafficking (14, 45–50). However, whereas numerous host cellular transport factors are known to promote endosome-to-Golgi apparatus transport of ricin toxins and SLT, such as the retromer complex, syntaxin 5 and 16, EpsinR, Rab6a, and clathrin, the roles of these factors in polyomavirus entry are not known (51–53, 57, 58). Additionally, transport to late endosomes is known to be important for polyomaviruses, but it is not known whether cellular retrograde transport factors involved in transport from late endosomes, such as Rab9 and Tip47, are important for polyomavirus infectivity. Future work examining the role of these host factors in polyomavirus infection and determining what cellular host factor retro-2\textsuperscript{cycl} binds will aid our understanding of how polyomaviruses and toxins undergo retrograde trafficking.

It is unlikely that retro-2\textsuperscript{cycl} binds to polyomaviruses directly and therefore decreases the likelihood of escape mutations, since infectious mutants would have to use alternate trafficking pathways to enter cells. Since the majority of people are persistently infected with JCPyV and BKPyV (2, 7, 54–56), the ability of retro-2\textsuperscript{cycl} to reduce the spreading of JCPyV, BKPyV, and SV40 in established infections suggests that these compounds may control viral dissemination in previously infected individuals. Further optimization of retro-2\textsuperscript{cycl} may result in effective antiviral therapies to treat or prevent diseases caused by human polyomaviruses or other pathogens that use retrograde trafficking during infection.

MATERIALS AND METHODS

Cells, viruses, plasmids, and antibodies. For all of the details of the cells, viruses, plasmids, and antibodies used in this study, see Text S1 (Materials and Methods) in the supplemental material.

Retro-2\textsuperscript{cycl} inhibition of infection. For all of the details of the infection studies described here, including dose-dependent inhibition of infection by retro-2\textsuperscript{cycl}, time course experiments, multicycle growth assays, and cell tropism experiments, see Text S1 (Materials and Methods) in the supplemental material.

Flow cytometry. For all of the details of the flow cytometry done in this study, including scoring of viral infectivity and binding assays, see Text S1 (Materials and Methods) in the supplemental material.

Viability assays. For all of the details of the viability assays done in this study, see Text S1 (Materials and Methods) in the supplemental material.

Virus purification and labeling. For all of the details of the virus purification and labeling done in this study, as well as details of pseudovirus production, see Text S1 (Materials and Methods) in the supplemental material.

Retro compounds. For all of the details of the synthesis of retro-2\textsuperscript{cycl} and its chemical derivatives, as well as X-ray crystallography and the deposition of X-ray structures, see Text S1 (Materials and Methods) in the supplemental material.

Microscopy. For all of the details of the virus purification and labeling done in this study, as well as details of PLAs, see Text S1 (Materials and Methods) in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00729-13/-/DCSupplemental.

Text S1, DOCX file, 0.1 MB.
Figure S1, TIF file, 0.5 MB.
Figure S2, TIF file, 0.9 MB.
Figure S3, TIF file, 0.9 MB.
Figure S4, TIF file, 1.2 MB.
Figure S5, TIF file, 1.2 MB.
Figure S6, TIF file, 7 MB.
Figure S7, TIF file, 1.5 MB.

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REFERENCES

1. Jiang M, Abend IR, Johnson SF, Imperiale MJ. 2009. The role of polyomaviruses in human disease. Virology 384:266–273.

2. Kean JM, Rao S, Wang M, Garcea RL. 2009. Seroprevalence of human polyomaviruses. PLoS Pathog. 5:e1000363. doi: 10.1371/journal.ppat.1000363.

3. Monaco MC, Atwood WJ, Gravel M, Tornatore CS, Major EO. 1996. JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency. J. Virol. 70:7004–7012.

4. Monaco MC, Jensen PN, Hou J, Durham LC, Major EO. 1998. Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. J. Virol. 72:9918–9923.

5. Ferencyz MW, Marshall LJ, Nelson CD, Atwood WJ, Nath A, Khalili K, Major EO. 2012. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin. Microbiol. Rev. 25:471–506.

6. Shinhoara T, Matsuda M, Cheng SH, Marshall J, Fujita M, Nagashima K. 1993. BK virus infection of the human urinary tract. J. Med. Virol. 41:301–305.

7. Knowles WA, Pipkin P, Andrews N, Vyse A, Minor P, Brown DW, Miller E. 2003. Population-based study of antibody to the human polyomaviruses BKV and JCV in the same polyomavirus SV40. J. Med. Virol. 71:115–123.

8. Kitamura T, Aso Y, Kuniyoshi N, Hara K, Yogo Y. 1990. High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. J. Infect. Dis. 161:1128–1133.

9. Zurhein G, Chou SM. 1965. Particles resembling papova viruses in human cerebral demyelinating disease. Science 148:1477–1479.

10. Boothpur R, Brennan DC. 2010. Human polyoma viruses and disease with emphasis on clinical BK and JC. J. Clin. Virol. 47:306–312.

11. Binet I, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, Querbes W, O’Hara BA, Williams G, Atwood WJ. 2013. A lipid receptor sorts polyomavirus in human tonsil tissue: implications for viral latency. J. Virol. 89:513–521.

12. Engel S, Heger T, Mancini R, Herzog F, Kartenbeck J, Hayer A, Querbes W, O’Hara BA, Williams G, Atwood WJ. 2013. Inhibitors of the polyomavirus entry into host cells. Cell 154:384–400.

13. Querbes W, O’Hara BA, Williams G, Atwood WJ. 2006. Invasion of host cells by JC virus identifies a novel role for caveolae in endosomal sorting of noncaveolar ligands. J. Virol. 80:9402–9413.

14. Qian M, Cai D, Verhey KJ, Tsai B. 2009. A lipid receptor sorts polyomavirus from the endolysosome to the endoplasmic reticulum to cause infection. J. Virol. 85:4198–4211.

15. Querbes W, O’Hara BA, Williams G, Atwood WJ. 2006. Invasion of host cells by JC virus identifies a novel role for caveolae in endosomal sorting of noncaveolar ligands. J. Virol. 80:9402–9413.

16. Engel S, Heger T, Mancini R, Herzog F, Kartenbeck J, Hayer A, Helenius A. 2011. The role of endosomes in SV40 entry and infection. J. Virol. 85:4198–4211.

17. Querbes W, O’Hara BA, Williams G, Atwood WJ. 2006. Invasion of host cells by JC virus identifies a novel role for caveolae in endosomal sorting of noncaveolar ligands. J. Virol. 80:9402–9413.

18. Qian M, Cai D, Verhey KJ, Tsai B. 2009. A lipid receptor sorts polyomavirus from the endolysosome to the endoplasmic reticulum to cause infection. J. Virol. 85:4198–4211.

19. Schelhaas MA, Malmström J, Pelkmans L, Haugstetter J, Ellgaard L, Grünewald K, Helenius A. 2007. Simian virus 40 depends on ER protein folding and quality control factors for entry into host cells. Cell 131:516–529.

20. Lilley BN, Gilbert JM, Ploegh HL, Benjamin TL. 2006. Murine polyomavirus requires the endoplasmic reticulum protein derlin-2 to initiate infection. J. Virol. 80:8739–8744.

21. Magnuson B, Rainey EK, Benjamin T, Baryshev M, Mkrchian S, Tsai B. 2005. ERp29 triggers a conformational change in polyomavirus to stimulate membrane binding. Mol. Cell 20:289–300.

22. Rainey-Barger KE, Magnuson B, Tsai B. 2007. A chaperone-activated nonenveloped virus perforates the physiologically relevant endoplasmic reticulum membrane. J. Virol. 81:12996–13004.

23. Goodwin EC, Lipovsky A, Inoue T, Magaldi TG, Edwards AP, Van Goor KE, Paton AW, Paton JC, Atwood WJ, Tsai B, Dimiao D. 2011. BiP and multiple DNAJ molecular chaperones in the endoplasmic reticulum are required for efficient simian virus 40 infection. mBio 2:e00101–11. doi: 10.1128/mBio.00101-11.

24. Stechmann B, Bai SK, Gobbo E, Lopez R, Merer G, Pinchard S, Panigai L, Tenza D, Raposo G, Beaumelle B, Sauvade R, Gillet D, Johannes L, Barbier J. 2010. Inhibition of retrograde transport protects mice from lethal ricin challenge. Cell 141:231–242.

25. Lipovsky A, Popa A, Pimienta G, Wyler M, Bhan A, Kuruvilla L, Guie MA, Poffenberger AC, Nelson CD, Atwood WJ, Dimiao D. 2013. Genome-wide siRNA screen identifies the retromer as a cellular entry factor for human papillomavirus. Proc. Natl. Acad. Sci. U. S. A. 110: 7452–7457.
48. Tsai B, Gilbert JM, Stehle T, Lencer W, Benjamin TL, Rapoport TA. 2003. Gangliosides are receptors for murine polyoma virus and SV40. EMBO J. 22:4346–4355.
49. Gorelik L, Reid C, Testa M, Brickelmaier M, Bossolasco S, Pazzi A, Bestetti A, Carmillo P, Wilson E, McAuliffe M, Tonkin C, Carulli JP, Lugovskoy A, Lazzarin A, Sunyaev S, Simon K, Cinque P. 2011. Progressive multifocal leukoencephalopathy (PML) development is associated with mutations in JC virus capsid protein VP1 that change its receptor specificity. J. Infect. Dis. 204:103–114.
50. Sandvig K, Spilsberg B, Lauvrak SU, Torgersen ML, Iversen TG, van Deurs B. 2004. Pathways followed by protein toxins into cells. Int. J. Med. Microbiol. 293:483–490.
51. Johannes L, Popoff V. 2008. Tracing the retrograde route in protein trafficking. Cell 135:1175–1187.
52. Popoff V, Mardones GA, Tenza D, Rojas R, Lamaze C, Bonifacino JS, Raposo G, Johannes L. 2007. The retromer complex and clathrin define an early endosomal retrograde exit site. J. Cell Sci. 120:2022–2031.
53. Bonifacino JS, Hurley JH. 2008. Retromer. Curr. Opin. Cell Biol. 20:427–436.
54. Chesters PM, Heritage J, McCance DJ. 1983. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J. Infect. Dis. 147:676–684.
55. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH. 2009. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J. Infect. Dis. 199:837–846.
56. Knowles WA. 2006. Discovery and epidemiology of the human polyomaviruses BK virus (BKV) and JC virus (JCV). Adv. Exp. Med. Biol. 577:19–45.
57. Mallard F, Tang B, Galli T, Tenza DL, Saint-Pol AS, Yuem X, Antony G, Hong W, Goud B, Johannes L. 2002. Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. J. Cell Biol. 156:653–664.
58. Amessou M, Fradagada A, Falguières T, Lord JM, Smith DC, Roberts LM, Lamaze C, Johannes L. 2007. Syntaxin 16 and syntaxin 5 are required for efficient retrograde transport of several exogenous and endogenous cargo proteins. J. Cell Sci. 120:1457–1468.