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Journal Title: Journal of Virology
Volume: Volume 84, Number 9
Publisher: American Society for Microbiology (ASM) | 2010-05, Pages 4243-4251
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JVI.00129-10
Permanent URL: http://pid.emory.edu/ark:/25593/f937b

Final published version: http://jvi.asm.org/content/84/9/4243

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Accessed September 24, 2020 8:32 AM EDT
Abl Family Tyrosine Kinases Regulate Sialylated Ganglioside Receptors for Polyomavirus

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Received 20 January 2010/Accepted 17 February 2010

Sialylated lipids serve as cellular receptors for polyomaviruses. Using pharmacological inhibitors and cell lines derived from knockout mice, we demonstrate that Abl family tyrosine kinases are required for replication of mouse polyomavirus and BK virus, a human polyomavirus associated with allograft failure following kidney transplantation. We show that decreasing Abl family kinase activity results in low levels of cell surface ganglioside receptors for mouse polyomavirus and that inhibition of sialidase activity promotes virion binding in the absence of Abl family kinase activity. These data provide evidence that Abl family kinases reduce ganglioside turnover in the plasma membrane by inhibiting host cell sialidase activity. Thus, Abl family kinases regulate the susceptibility of cells to polyomavirus infection by modulating gangliosides required for viral attachment.

Polyomaviruses (PyVs) are ubiquitous silent pathogens that persist lifelong in a variety of vertebrate hosts, including humans, but that can become opportunistic pathogens in the setting of depressed immune function (21). Nephritis associated with human PyV BK infection is an important cause of dysfunction and loss of kidney transplants (44), and JC virus (JCV) is the etiologic agent for progressive multifocal leukoencephalopathy (PML), a demyelinating disease in AIDS patients and in patients receiving immunotherapies for autoimmune and inflammatory diseases (21) that is usually fatal. Recently, two new human PyVs have been discovered in respiratory tract infections and clonally integrated sequences of another novel PyV have been detected in an aggressive cutaneous malignant (4). There are currently no effective antiviral therapeutics for PyV infection.

PyVs are nonenveloped double-stranded DNA viruses that bind to cell surface sialylated ganglioside glycolipids and glycoproteins (11, 14–16, 29, 46, 51). Following virion adsorption to host cell receptors, the various PyV family members utilize similar intracellular trafficking pathways during infection. Virions are endocytosed via clathrin- or caveolin-dependent mechanisms, then traffic to endolysosomes before trafficking to the endoplasmic reticulum (ER), where virion disassembly is initiated (32, 39, 40). For mouse PyV (MPyV), the ganglioside GD1a serves as a receptor for viral binding but also for sorting virions from late endosomes and/or lysosomes to the ER (42).

Retrotranslocation from the ER to the cytosol results in further disassembly, with the viral protein-minichromosome complex then being transported across nuclear pores to the nucleus, where viral gene transcription, viral DNA replication, and progeny assembly occur.

Host tyrosine kinases have been implicated in PyV infection. Virus uptake activates protein tyrosine kinase(s) and induces a transient reorganization of the actin network (15, 17, 40). Experiments with the relatively nonspecific inhibitor genistein suggest that tyrosine kinases are required for internalization of simian virus 40 (SV40) PyV and for development of “actin tails” associated with vesicle-encased virions (40). In addition to SV40, genistein has also been shown to inhibit entry by JCV and BK virus (BKV) (12, 43), suggesting a role for tyrosine kinases in entry of many species of PyVs. However, little information regarding which tyrosine kinases mediate entry is available. Finally, the middle T (MT) antigen of MPyV is an integral membrane protein that binds to and is phosphorylated by Src family kinases, (9) thereby creating docking sites for SH2 domain-containing enzymes and adaptor proteins (18).

Abl family tyrosine kinases, which include Ab1 and Ab2, regulate cytoskeletal and trafficking functions in cells (50). Abl family kinases are mutated in human cancers such as chronic myelogenous leukemia (CML), and drugs such as STI-571 (imatinib mesylate; Gleevec), which inhibit Ab1 family kinases, have been developed as cancer therapeutics (19). Ab1 family kinases are also involved in the life cycles of several viral and bacterial pathogens (2). For example, Abl family tyrosine kinases contribute to entry into mammalian cells of Pseudomonas aeruginosa, Shigella flexneri, and Chlamydia trachomatis (5, 13, 41), although the precise mechanisms remain unclear. In addition, Abl family kinases mediate formation of actin-filled membranous protrusions, which are required for motility of pathogens such as poxviruses and enteropathogenic Escherichia coli on and between cells (45, 52), facilitating the spread...
of the infection. Our recent studies demonstrate that STI-571 can be used both as a prophylactic and as a therapeutic for orthopoxvirus infections (45; P. Reeves and D. Kalman, unpublished data).

The requirement for Abl family kinases in pathogenesis of diverse microbes led us to investigate their potential role in PyV infection. Using cell lines lacking Abl family kinases, together with the specific Abl inhibitor STI-571, we report here that Abl family kinases control the levels of ganglioside receptors in the plasma membrane by negatively regulating a plasma membrane sialidase.

MATERIALS AND METHODS

Cell culture, antibodies, and reagents. 3T3 cells and 3T3 cells derived from Ab1+/−/Ab2−/− mice (25) were maintained in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) as previously described (52). For BKV infections, primary human renal proximal tubule epithelial (RPTE) cells were cultured as described previously (22). Antibodies used were as follows: T-antigen (TAg) monoclonal antibody (MAb) F4, which recognizes all three MPyV TAg proteins, large T (LT), middle T (MT), and small T (ST) (38) for Western analysis of TAg production; polyclonal anti-TAg ascites fluid (kindly provided by L. T. Benjamin, Harvard Medical School) for immunofluorescent detection of MPyV LT+ nuclei; polyclonal antibody PAb416 for BKV TAg detection (20); and rat VPI MAb for neutralization of extracellular virus and immunofluorescent detection of MPyV virions. VPI MAb was generated by immunizing rats with MPyV VPI virus-like particles provided by R. Garcia (University of Colorado, Denver, CO), and anti-GD1a and anti-GT1b (Millipore) were used for analysis of cell surface gangliosides. The salt of the Abl family kinase inhibitor STI-571 was synthesized as described previously (48) and was used at 20 μM unless otherwise indicated. Sialidase inhibitor 2,3-dideoxy-2-deoxy-N-acetylneuraminic acid (DANA) was purchased from Sigma and was used at 100 μM unless otherwise indicated. Due to instability, a new DANA stock solution was prepared just prior to each experiment. Exogenous gangliosides GD1a, GD1b, GT1b, and GM1 were purchased from Matrex. Biotinylated Sambucus nigra lectin (SNA) and Maackia amurensis lectin II (MAL II) were purchased from Vector Labs.

Infections and drug treatments. Virus stocks of MPyV strain A2 or purified BKV strain Dunlop were prepared and titered as previously described (22, 31). For MPyV infections, cells were incubated with virus at a multiplicity of infection (MOI) of 5 PFU/cell for 1 h at 37°C. Virus was removed, and extracellular virions were neutralized by the addition of the VPI MAb in DMEM–2% calf serum for 30 min at 37°C. Following neutralization of extracellular virions, the medium was exchanged with DMEM–10% FBS, and internalized virus was allowed to replicate for 24 h. For BKV infections, RPTE cells were infected with purified BKV at a MOI of 0.5 infectious unit (IU)/cell as previously described (22) and allowed to replicate for 48 h.

For pretreatment with STI-571 and/or DANA, cells were incubated in medium containing the drug for 16 to 24 h prior to infection. Drug-containing medium was removed, and cells were washed prior to addition of virus. Drug added during viral adsorption was added directly to the viral lysate for the 1-h incubation period and was removed prior to neutralization of extracellular virions. Drug added posttreatment was added to medium only after the neutralization of extracellular virions and was maintained for the subsequent infection period. Previous reports suggest that inhibitors of Abl family kinases act within minutes after addition to tissue culture media and are effective for days without replenishment (3, 10, 45, 52). Following the various treatments, the level of infection was determined by assaying for TAg production in infected cells by immunofluorescence microscopy or Western analysis as described below.

TAg analysis and quantitation. TAg production was assessed by both Western blotting and immunofluorescence microscopy. For Western analysis of MPyV-infected cells, samples were lysed and processed as previously described (30) and probed with F4 antibody. Equivalent loading of samples was verified by detection of a non-MPyV-related protein that is nonspecifically recognized by the F4 antibody and that is present in equal amounts in all samples, both infected and uninfected. For BKV-infected cells, total cellular protein was harvested for Western analysis as previously described (22) and data were quantified using a Typhoon imaging system and the ImageQuant software (GE Healthcare). A cell metabolism WST-1 assay was performed according to the manufacturer’s instructions (cell proliferation reagent WST-1; Roche Applied Sciences) to measure the cytotoxicity of STI-571.

For immunofluorescence detection of TAg following MPyV infection, cells plated on glass coverslips were fixed and stained as described elsewhere (23) using a MAB to LT to detect LT+ nuclei. Images were acquired with a scientific-grade cooled charge-coupled device (Cool-Snap HQ ORCA-ER chip) on a multiwavelength, wide-field, three-dimensional microscopy system (Intelligent Imaging Innovations, Denver, CO), based on a 200 M inverted microscope (Carl Zeiss, Thornwood, NY). For each condition or experiment, image data were collected on the same day using identical exposure times. LT+ nuclei were counted, as were total nuclei, as determined by DAPI (4′,6-diamidino-2-phenylindole) staining, and data are presented as the percentage of total nuclei counted that were LT+. Duplicate samples were counted for each condition, a minimum of 300 nuclei per coverslip per experiment. On average, approximately 35% of total nuclei were TAg positive per coverslip under control conditions. In all cases, data were normalized to represent the percentage of TAg-positive nuclei in control 3T3 cells as 100%.

Cell surface GD1α analysis. The cell surface GD1α ganglioside was visualized using GD1α MAb and immunofluorescence microscopy as previously described (49). Brieﬂy, cells were incubated with GD1α antibody (1:250 dilution) in Hanks balanced salt solution (HBSS) and 3% bovine serum albumin (BSA) for 30 min at 37°C prior to ﬁxation. Cells were then washed and ﬁxed, and subsequent ﬂuorescent staining was performed under nonpermeabilizing conditions to recognize only the cell surface ganglioside. Images were acquired as described for TAg analysis, and expression of GD1α was quantitated using the MASK function in the Intelligent Imaging Innovations software package. Details regarding use of the MASK function to determine the quantitation of colocalization of overlapping fluorescent signals have been described elsewhere (53). Brieﬂy, eight images were collected from each slip, under identical acquisition parameters, and intensity contrast parameters were adjusted to be equivalent across all images. Next, the area of GD1α-positive signal that overlapped with actin cytoskeletal staining, a representation of total cell surface area, was calculated for each image. Data are presented as the percentage of total cell surface area (in μm²) that was GD1α positive. Duplicate coverslips were assessed for each condition. In all cases, data were normalized such that the percentage of GD1α expression in control 3T3 cells was represented as 100%.

To verify the speciﬁcity of anti-GD1α staining, 3T3 cells were treated prior to addition of antibody with sialidase, which cleaves terminal sialic acid moieties, thus converting GD1α to GM1, which is not recognized by the GD1α antibody. 3T3 cells treated with neuraminidase no longer showed any signal following staining with anti-GD1α (data not shown). In addition, 3T3 cells supplemented with exogenous ganglioside GD1α (the method is described below) showed an ~2-fold increase in GD1α-positive surface area compared to mock-treated 3T3 cells, whereas GM1-supplemented cells showed no increase in GD1α staining compared to mock-treated cells. Taken together, these data conﬁrm the speciﬁcity of the GD1α antibody for ganglioside GD1α.

MPyV binding assay. Cells were incubated with virus in HEPES buffered medium for 30 min at 4°C to allow viral binding but inhibit endocytosis. Following binding, cells were washed extensively with cold phosphate-buffered saline (PBS) to remove unbound virions and fixed at 4°C. Cells were subsequently stained with VPI MAb under nonpermeablizing conditions to detect bound virions on the surface of the cells and assessed by immunofluorescence microscopy.

Supplementation with exogenous gangliosides. Cells were supplemented with exogenous gangliosides at 50 μg/ml for 16 to 24 h in serum-free DMEM. Unincorporated ganglioside was removed by washing extensively with DMEM–20% FBS prior to analysis or infection.

Flow cytometry and lectin analysis. Cells were stained with biotinylated lectins (either MAL II or SNA at 0.1 μg/ml) and streptavidin-allophycocyanin (APC)-conjugated tetramers as previously described (24). Samples were immediately acquired on a FACS Calibur (BD Biosciences), and data were analyzed using CellQuest software (BD Biosciences).

RESULTS

Abl family kinases are necessary for PyV infection. Abl family tyrosine kinases have recently been found to play a crucial role in a wide variety of viral and bacterial infections (2). To determine whether Abl family kinases are involved in MPyV infection, we utilized cell lines derived from mice lacking both c-Ab1 and e-Ab2 (Ab1−/−/Ab2−/−). At 24 h postinfection, indirect immunofluorescence microscopy showed that ~10-fold fewer Ab1−/−/Ab2−/− cells had LT+ nuclei than did
wild-type 3T3 cells (Fig. 1A). This difference in levels of T-antigen expression was confirmed by Western analysis (Fig. 1B). Bands representing the three T-antigen proteins LT, MT, and ST were barely detectable in infected \( Abl1^{-/-} \) \( Abl2^{-/-} \) cells (Fig. 1B). This dramatic decrease in T-antigen production in infected \( Abl1^{-/-} \) \( Abl2^{-/-} \) fibroblasts suggests that Abl family kinases play a role in MPyV infection. Using 3T3 cells derived from mice lacking either c-Abl1 or c-Abl2 individually, we found that either of these Abl family kinases is sufficient to support MPyV infection (data not shown). These findings indicate that Abl1 and Abl2 act redundantly to support MPyV infection.

To confirm these results, we next assessed the effect of STI-571, an inhibitor of Abl family kinases, on MPyV infection. Treatment of 3T3 cells with STI-571 16 h before and throughout the 24-h infection period decreased numbers of LT-expressing cells 10-fold compared to numbers of mock-treated 3T3 cells (Fig. 2A). Similar effects were evident in primary baby mouse kidney epithelial cells (data not shown). To determine whether Abl family kinases act during early or late steps in PyV infection, 3T3 cells were pretreated with STI-571 for 16 h prior to adsorption of virus, only during the 1-h adsorption period, or only after removal of the viral lysate. As shown in Fig. 2B, the infectivity of MPyV was decreased only in those

![FIG. 1. Abl1^{-/-} Abl2^{-/-} cells are nonpermissive to infection with MPyV. (A) Immunofluorescence images and quantitation of LT^{+} nuclei in 3T3 cells and Abl1^{-/-} Abl2^{-/-} cells infected with MPyV and assessed for TAg production 24 h postinfection. Cells are stained with TAg antibody (Ab) to visualize infected cells (red), DAPI to visualize cell nuclei (blue), and Alexa-488–phalloidin to recognize actin (green). Scale bars, 50 \( \mu \)m. Quantitation of LT^{+} nuclei was determined as outlined in Materials and Methods, and all values are normalized to infected 3T3 control cells as 100%. (B) Western analysis of 3T3 cells and Abl1^{-/-} Abl2^{-/-} cells infected with MPyV or mock infected and assessed for T-antigen proteins LT, MT, and ST at 24 h postinfection. Equivalent loading of samples was verified by detection of a non-PyV-specific band (*), present in all samples, that was recognized by the F4 TAg Ab.](image)

![FIG. 2. 3T3 cells pretreated with STI-571 are less permissive to infection by MPyV. (A) Immunofluorescence images and quantitation of LT^{+} nuclei in 3T3 cells continuously exposed to 20 \( \mu \)M STI-571 for 16 h before infection with MPyV and fixed 24 h after infection. Cells are stained as described for Fig. 1. Scale bars, 50 \( \mu \)m. (B) Quantitation of LT^{+} nuclei in cells pretreated with STI-571 for 16 h prior to adsorption of virus (Pre), only during viral adsorption (Ads), or only during the 24-h infection period following removal of the viral lysate (Post). (C) Western analysis of 3T3 cells pretreated with STI-571 for 16 h prior to infection or left untreated and assessed for TAg production 24 h postinfection. Equivalent loading of samples was verified by detection of a non-PyV-specific band (*), present in all samples, that was recognized by the F4 TAg Ab. (D) Quantitation of LT^{+} nuclei in 3T3 cells pretreated for 16 h with increasing concentrations of STI-571 (0 to 20 \( \mu \)M) prior to infection with MPyV. (E) Quantitation of LT^{+} nuclei in 3T3 cells pretreated with 20 \( \mu \)M STI-571 for increasing periods of time (0 to 16 h) prior to infection with MPyV. Quantitation of LT^{+} nuclei in panels A, B, D, and E was determined as outlined in Materials and Methods, and all values are normalized to infected 3T3 control cells as 100%.](image)
cells exposed to STI-571 using the pretreatment protocol compared to that in mock-treated control cells. Western analysis of pretreated cells confirmed this result (Fig. 2C). By contrast, STI-571 did not effectively inhibit MPyV infection when added to cells during or after virion adsorption.

Using the 16-h pretreatment regimen, we next determined the concentration of STI-571 required to reduce MPyV infection by 50% (IC50). As shown in Fig. 2D, there was an inverse correlation between STI-571 concentration and frequency of LT+ cells, with an IC50 of ~5 μM. We next determined the duration of pretreatment required to reduce the percentage of LT+ cells by 50% (t1/2) at an STI-571 concentration of 20 μM (Fig. 2E). Although infectivity for MPyV decreased after 2 to 4 h of pretreatment, with a t1/2 of ~5 h, at least 8 h of pretreatment was required to reduce the percentage of LT+ cells by 75% compared to mock-treated cells. We could find no evidence of toxicity upon treatment of 3T3 cells or other cell types (e.g., Rpte cells [see Fig. 7]) with STI-571 at concentrations as high as 40 μM. Taken together, these data indicate that Abl family kinases affect a cellular process(es) involved in early steps of MPyV infection.

**Abl family kinases regulate binding of MPyV virions to cells.** We next determined whether Abl family kinases affected virion adsorption to cells. To do this, we utilized indirect immunofluorescence microscopy together with a monoclonal antibody against VP1, the major PyV capsid protein, to visualize virions at the membrane surface immediately following binding. Cells were held at 4°C during adsorption to inhibit viral uptake and then fixed and stained with VP1 MAb to detect surface virions. As shown in Fig. 3, diffuse VP1 staining was evident on the surfaces of infected, unpermeabilized 3T3 cells. In contrast, little VP1 staining was detected at the surface in either Abl1−/− Abl2−/− cells or 3T3 cells pretreated with STI-571. Together, these data indicate that Abl family kinases are required for binding of virions to the plasma membrane.

**Abl family kinases regulate cell surface levels of ganglioside GD1a.** We next assessed cell surface levels for the two known MPyV receptors, the gangliosides GD1a and GT1b, by indirect immunofluorescence microscopy. In uninfected wild-type 3T3 cells, no signal above background was evident upon staining with anti-GT1b, indicating that these cells likely do not express this ganglioside at detectable levels (data not shown). Although anti-GD1a staining was heterogeneous (Fig. 4A), on average GD1a was detectable on ~30% of the total cell surface area of 3T3 cell monolayers (normalized to 100% in the graph in Fig. 4A). By contrast, little or no GD1a was expressed by 3T3 cells pretreated with STI-571 or by Abl1−/− Abl2−/− cells (Fig. 4A). To determine the IC50 of STI-571 for GD1a loss, 3T3 cells were exposed to increasing concentrations of STI-571, from 0 to 20 μM, for 16 h and assessed for level of reduction in cell surface GD1a. Notably, the resulting dose-response curve (Fig. 4B, red) nearly matched that generated for T-antigen production in infected 3T3 cells after STI-571 pretreatment (Fig. 4B, black [data reproduced from Fig. 2D]), with an IC50 of ~5 μM calculated for each curve. Similar profiles of GD1a (Fig. 4C, red) and T-antigen expression (Fig. 4C, black [reproduced from Fig. 2E]) were seen when the duration of STI-571 pretreatment was varied. It is also interesting to note that the loss of surface GD1a induced by STI-571 slightly preceded the decrease in T-antigen production (t1/2 of 3 h for GD1a and 5 h for T antigen).

**Exogenous GD1a renders Abl kinase-deficient cells susceptible to MPyV infection.** Exogenous gangliosides have been shown to restore infectibility to cell lines lacking the endogenous PyV ganglioside receptor (15, 16, 29, 51). Thus, we asked whether exogenous gangliosides would render Abl1−/− Abl2−/− cells or STI-571-treated wild-type 3T3 cells infectious by MPyV. Abl1−/− Abl2−/− cells or 3T3 cells pretreated with STI-571 were exposed for 16 h to GD1a or, as a negative control, to GM1 and infected with MPyV. Abl1−/− Abl2−/− cells treated with exogenous GD1a supported MPyV infection to almost the same level as 3T3 cells (Fig. 5A). In addition, exogenous GD1a partially restored the infectibility of 3T3 cells pretreated with STI-571 (Fig. 5B). Taken together, these data indicate that Abl1−/− Abl2−/− cells are not intrinsically non-permissive for MPyV infection and that GD1a is sufficient to restore the susceptibility to infection by MPyV of cells lacking Abl family kinase activity.
Abl family kinases regulate cell surface sialidase activity. Primate, murine, and human PyVs bind to receptors with a terminal sialic acid(s) linked to galactose (37). The sialylation state of plasma membrane glycolipids and glycoproteins is regulated by sialyltransferases within the Golgi apparatus that add sialic acid moieties to precursors and by sialidases in the plasma membrane, lysosome, and cytosol that remove them (33–35, 55, 56). We hypothesized that Abl family kinases might negatively regulate one or more cellular sialidases and thereby control plasma membrane levels of gangliosides. Alternatively, Abl family kinases may activate sialyltransferases. To distinguish between these alternatives, we first assessed whether Abl family kinases regulate sialyltransferases. To assess cell surface sialylation, cells were stained with fluorescently labeled Maackia amurensis lectin II (MAL II) or Sambucus nigra lectin (SNA) and analyzed by flow cytometry. MAL II and SNA recognize 2,3- and 2,6-sialic acid linkages, respectively, on both gangliosides and glycoproteins. No significant differences in lectin binding between Abl1−/− Abl2−/− cells and control cells, or between wild-type cells incubated with or without STI-571 for 24 h, were evident (data not shown). Moreover, pretreatment of MDCK cells with STI-571 had no detectable effects on infectivity of influenza virus H1N1 (K. Bradley, D. Steinhauer, and D. Kalman, unpublished data), which binds to sialic acid residues on glycoproteins (7). Together, these data suggest that sialyltransferase activity is not regulated by Abl family kinases.

We next assessed whether inhibition of plasma membrane sialidase activity was sufficient to restore GD1a levels in wild-type cells treated with STI-571 or in Abl1−/− Abl2−/− cells. Plasma membrane sialidase preferentially hydrolyzes gangliosides and demonstrates minimal activity against glycoproteins and oligosaccharides (35). 2,3-Didehydro-2-deoxy-N-acetylneuraminic acid (DANA) is a relatively cell-impermeable sialic acid transition state analog that primarily inhibits the activity of plasma membrane sialidase when added to intact cells in culture (26). Exposure of Abl1−/− Abl2−/− cells to DANA increased expression of cell surface GD1a to nearly wild-type levels (Fig. 6A) and increased infectibility by 4-fold compared to expression by mock-treated cells (Fig. 6B). The effect was concentration dependent. Notably, although infectibility was restored with DANA, the percentage of LT− cells was only 25% of that seen in 3T3 cells. Pretreatment of 3T3 cells with 100 μM DANA alone did not lead to any significant increase in either GD1a expression or infectibility over those for untreated cells (data not shown); however, pretreatment of 3T3...
cells with DANA and STI-571 together resulted in levels of GD1a on the cell surface that were ~6-fold higher than seen on cells pretreated with STI-571 alone (Fig. 6C). Moreover, cells exposed to both DANA and STI-571 exhibited ~7-fold higher susceptibility to infection by MPyV than cells pretreated with STI-571 alone (Fig. 6D). However, as with Abl1−/−Abl2−/− cells, susceptibility to infection by PyV was only partially restored compared to that of wild-type 3T3 cells. Because treatment with DANA restored plasma membrane GD1a in Abl1−/−Abl2−/− cells and STI-571-treated 3T3 cells to levels similar to those seen in 3T3 cells, these data demonstrate that Abl family kinases regulate plasma membrane sialidase activity. Notably, the ability of Abl1−/−Abl2−/− cells to express cell surface GD1a following inhibition of plasma membrane sialidase offers further evidence that Abl kinases do not regulate sialyltransferase activity.

Abl family kinases mediate BKV infectivity. To test relevance to a human pathogen, we next asked whether Abl family kinases regulate the susceptibility to BKV infection of primary human renal proximal tubule epithelial (RTPE) cells. BKV is known to use gangliosides GD1a and GT1b, both of which contain an α-2,8-linked disialic acid motif, as cellular receptors (29). Expression of BKV LT was reduced by ~85% upon pretreatment of RTPE cells with up to 40 μM STI-571 (Fig. 7A) without cytotoxic effects (Fig. 7B). Addition of GD1a, but not GT1b or GM1, restored the infectibility of STI-571-treated cells to a limited, but statistically significant extent (up to 2-fold) (Fig. 7C). These data suggest that regulation of ganglioside receptors by Abl family kinases occurs in human cells and can control infection by human PyVs.

DISCUSSION

Human PyV infections are associated with kidney transplant rejection and PML, a demyelinating disease seen in patients immunocompromised by HIV/AIDS or by agents that block T-cell entry into the central nervous system (CNS). The substantial morbidity and mortality of these PyV-associated diseases highlight the pressing need for effective therapeutic strategies. In this study, we provide evidence that Abl family tyrosine kinases modulate levels of cell surface gangliosides, sialylated glycolipids that serve as receptors for PyV. Abl family tyrosine kinases have been implicated in regulating the life cycles of a number of bacterial and viral pathogens, raising the possibility that inhibitors of Abl family kinases (e.g., STI-571) may prove effective in treating PyV-associated diseases in humans.

Gangliosides are amphipathic glycosphingolipids that consist of a ceramide anchor linked to an oligosaccharide chain of variable length and are distinguished by the presence of one or more sialic acid residues (1). The levels and diversity of gangliosides on the cell surface reflect a complex balance between ganglioside biosynthesis and degradation. Ganglioside biosynthesis is regulated by glycosyltransferases and sialyltransferases in the Golgi apparatus and ER, which sequentially add monosaccharides and sialic acids to ceramide to create continu-
Ganglioside degradation occurs by endocytosis and lysosomal degradation, as well as by the catalytic removal of sialic acids by sialidases within the plasma membrane, converting membrane-bound multisialogangliosides, such as GD1a, to less-complex monosialo- or asialogangliosides, such as GM1 (56). Our observations showing that cells lacking Abl1 and Abl2, which do not express GD1a on their surfaces, can be made to do so when sialidase activity is inhibited with DANA (Fig. 6A) suggest that Abl family kinases do not affect ganglioside biosynthesis pathways and likely do not regulate sialyltransferases, which sialylate both glycoproteins and glycolipids (54). The lack of any changes in lectin binding in cells lacking Abl family kinase activity (this report) and the inability of STI-571 to block influenza virus infection (data not shown) also support this conclusion. The data presented here provide evidence that Abl family kinases regulate cell surface ganglioside levels by controlling ganglioside-specific sialidase activity at the plasma membrane. Although a connection between Abl family kinases and regulation of sialidases has not previously been identified, our model is consistent with known activities of gangliosides and Abl family kinases in other systems. For example, inhibition of Abl family kinase activity in BCR-ABL-overexpressing leukemic cells results in increased cell surface expression of ganglioside GM1 (6), the direct downstream product of GD1a desialylation. GM1 is not a substrate for plasma membrane sialidase and therefore can accumulate in some tissues and cells as a result of upregulated sialidase activity at the plasma membrane (27, 47).

FIG. 6. Inhibition of cell surface sialidase activity in Abl kinase-deficient cells restores surface expression of ganglioside GD1a and increases susceptibility to infection by MPyV. (A) Immunofluorescence images and quantitation of cell surface GD1a in Abl1−/− Abl2−/− cells pretreated with DANA for 16 h. Quantitation represents the increase in cell surface GD1a expression following pretreatment with increasing concentrations of DANA (0 to 100 μM) in Abl1−/− Abl2−/− cells compared to untreated control 3T3 cells. (B) Immunofluorescence images and quantitation of LT+ nuclei in Abl1−/− Abl2−/− cells pretreated with DANA for 16 h prior to infection with MPyV. (C) Immunofluorescence images and quantitation of cell surface GD1a in 3T3 cells pretreated with STI-571 and DANA for 16 h. (D) Immunofluorescence images and quantitation of LT+ nuclei in 3T3 cells pretreated with STI-571 and DANA for 16 h prior to infection with MPyV. Scale bars (all images), 50 μm. Quantitation of GD1a and LT+ nuclei is normalized to infected 3T3 control cells as 100%.
transported directly to the ER (28). Because gangliosides are involved in virion trafficking to the ER (42), it remains possible that Abl family kinases regulate sialylation of gangliosides following virion uptake. In this regard, our preliminary data suggest that levels of LT were reduced by STI-571 added postinfection to RPTE cells infected with BKV or macrophages infected with MPyV (data not shown). These data raise the possibility that Abl family kinases mediate additional, postadsorption steps in PyV infection in a cell-type-dependent manner.

The question arises as to whether inhibitors of Abl family kinases such as STI-571 will prove useful as therapeutics for human PyVs. STI-571 has somewhat limited side effects, though some cardiotoxicity has been reported with long-term administration. Moreover, STI-571 does not have significant immunotoxicity, and it has been used both prophylactically and in a therapeutic context against poxvirus infections in mice (45; Reeves and Kalman, unpublished). However, it is unlikely that STI-571 will downregulate all ganglioside receptors on all cell types. In preliminary experiments, we have not detected significant reduction in viral load in mice acutely infected by MPyV (data not shown). However, in situations where viral infection is low level, as in the persistent phase of PyV infection, a decrease in ganglioside receptor availability may confer resistance to permissive host cells. Moreover, the capacity of STI-571 to act at a step following adsorption makes it a promising candidate therapeutic for BKV infection of kidney epithelial cells, a major host cell target in BKV-associated nephropathy in transplant patients.

ACKNOWLEDGMENTS

We thank Patrick Reeves and Bettina Bommarius for helpful discussions, Annette Hadley for technical assistance, Tony Koleske for Abl/Arg cells, and T. L. Benjamin for rat anti-LT ascites fluid. This work was supported by grants R21AI073317 (to A.E.L. and D.K.), R01AI056067 (to D.K.), R01CA71971 (to A.E.L.), and R01AI060584 (to M.J.I.) and American Heart Association Postdoctoral Fellowship 0825806G (to M.J.).

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