MEK1/2 Inhibitors Block Basal and Transforming Growth Factor 
β1-Stimulated JC Virus Multiplication

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The multiplication of the human neurotropic polyomavirus JC virus (JCV) is regulated by cell membrane receptors and nuclear transcription factors. Signaling pathways also play a role in determining the extent to which JCV can productively infect cells. These data show that constitutively active MEK1 protein (CA-MEK1), overexpressed in cultures of human glia, supports a substantial increase in late JCV protein (Vp-1) synthesis. The specificity of this pathway was indicated by no significant enhancement of JCV multiplication through activation of other components of mitogen-activated protein kinase pathways such as p38, Jun N-terminal protein kinase, and protein kinase A. Further evidence supporting the importance of signaling in JCV infection came from addition of transforming growth factor β1 (TGF-β1), which stimulated a 200% increase of Vp-1 expression. Specific MEK1/2 inhibitors, flavenoid PD98059 and U0126, decreased the basal and TGF-β1-stimulated Vp-1 expression by 95% or more. TGF-β1 is known to phosphorylate/activate Smad DNA binding proteins that could subsequently bind or increase binding to JCV promoter sequences, linking the effects of signaling with JCV transcriptional regulation. The effectiveness with which MEK1/2 inhibitors block JCV multiplication provides insight that may contribute to development of compounds directed against JCV.

Mammalian viruses are known to exploit host cell signaling machinery to regulate replication and host gene responses. The mitogen-activated protein kinase (MAPK) signaling pathway appears to facilitate such viral activity through a family of proline-directed serine-threonine proteins which normally function to transduce signals from the cell membrane to the nucleus in response to diverse extracellular stimuli (2, 14). This family of MAPK proteins consists of the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2, respectively [ERK1/2]), p38 MAPK (p38), c-Jun NH2-terminal kinase (JNK), and cyclin-dependent kinases (9).

The ERK pathway is the most thoroughly studied of cytoplasmic signaling pathways (4, 5). ERK1 (also known as p44 MAPK) and ERK2 (also known as p42 MAPK) play a central role in mediating cellular responses to a variety of signaling molecules (15). The activity states of ERK1/2 are regulated by the upstream proteins MAPK kinases 1 and 2 (MEK1/2). The activity states of MEK1/2 are, in turn, regulated by MAPK kinase kinases. MEK1/2 activate ERK1/2 by phosphorylating regulatory threonine and tyrosine residues. The activated ERK1/2 then translocate into the nucleus to participate in transcriptional regulation of target genes (4). Recently, regulatory roles for the ERK pathway have been implicated in regard to gene expression and replication of human cytomegalovirus (13), simian virus 40 (29), human immunodeficiency virus type 1 (HIV-1) (11, 31), coxsackievirus (17), and influenza virus (24).

The ERK1/2 as well as phosphatidylinositol 3-kinase and Rho family members can be activated by transforming growth factor β1 (TGF-β1), which mediates biological effects through their regulators. TGF-β mediates biological effects through interactions with at least three main TGF-β receptors, TβR1, TβR2, and TβR3/betaglycan (3, 16, 30). The intracellular signaling triggered by TGF-β involves, in part, the phosphorylation of Smad-related proteins which, in turn, transduce complex changes in the transcriptional regulation of various target genes.

Enam et al. proposed a hypothesis based on observations of high TGF-β1 levels accompanying HIV-1-associated progressive multifocal leukoencephalopathy (PML), the fatal JCV-mediated demyelination disease of the central nervous system (CNS) (7). The high levels of TGF-β1 as well as Smad3/4 in JCV-infected oligodendrocytes of the examined PML patient samples were observed by immunohistochemistry. Using chloramphenicol acetyltransferase cell culture assays, Enam et al. also showed activation of the JCV early and late promoters by Smad3/4 and suggested a proactive role for Smad3/4 in viral propagation.

We show here that addition of TGF-β1 to the culture medium of JCV-exposed cells stimulated JCV multiplication to levels higher than those of untreated controls. Also, inhibition of the MEK pathway, using the specific MEK1/2 inhibitors PD98059 (2’-amino-3’-methoxyflavonone; C16H13NO3) and U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminoethyl)butadiene; C15H16N2S2), resulted in significant decreases of JCV multiplication in both TGF-β1-stimulated and nonstimulated cultures. These findings point to stimulation of JCV multiplication by TGF-β1 occurring through the MEK pathway. These data are of interest considering that enhanced expression levels of TGF-β are reported to accompany immunosuppressive conditions, including AIDS. Therefore, targeting inhibition of the MEK1/2 pathway could be a promising strategy for the development of antiviral drugs.
TABLE 1. Effects of various signal transduction stimulants and inhibitors on JCV activity in PDA

<table>
<thead>
<tr>
<th>Chemical tested</th>
<th>Final conc</th>
<th>Action</th>
<th>% Vp-1†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>0</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Phorbol-12-myristate-13-acetate</td>
<td>100 ng/ml</td>
<td>Activates protein kinase C</td>
<td>100</td>
</tr>
<tr>
<td>Interleukin-β</td>
<td>1 ng/ml</td>
<td>Stimulates inflammatory and immune response</td>
<td>105</td>
</tr>
<tr>
<td>Tumor necrosis factor alpha</td>
<td>1 ng/ml</td>
<td>Activates JNK</td>
<td>110</td>
</tr>
<tr>
<td>Forskolin</td>
<td>5 μM</td>
<td>Activates adenylyl cyclase</td>
<td>110</td>
</tr>
<tr>
<td>Sphingosylphosphorylcholine</td>
<td>5 μM</td>
<td>Stimulates DNA binding activity of AP-1</td>
<td>110</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>1 μg/ml</td>
<td>Stimulates various immune defense mechanisms</td>
<td>200</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>5 ng/ml</td>
<td>Regulates proliferation, differentiation, and other cellular functions</td>
<td>200</td>
</tr>
<tr>
<td>Bisindolylmaleimide II</td>
<td>25 nM</td>
<td>Inhibits protein kinase C</td>
<td>100</td>
</tr>
<tr>
<td>H-89, dihydrochloride</td>
<td>100 nM</td>
<td>Inhibits protein kinase A</td>
<td>90</td>
</tr>
<tr>
<td>N-Nitro-l-arginine</td>
<td>100 μM</td>
<td>Inhibits nitric oxide synthases, bNOS and eNOS</td>
<td>90</td>
</tr>
<tr>
<td>SF600125</td>
<td>100 nM</td>
<td>Inhibits JNK</td>
<td>90</td>
</tr>
<tr>
<td>SP600125</td>
<td>1 μM</td>
<td>Inhibits p38 MAPK</td>
<td>90</td>
</tr>
<tr>
<td>PD98059</td>
<td>20 μM</td>
<td>Inhibits MEK1/2</td>
<td>5</td>
</tr>
<tr>
<td>U0126</td>
<td>10 μM</td>
<td>Inhibits MEK1/2</td>
<td>2</td>
</tr>
</tbody>
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† A measure of the viral activity observed in PDA cultures 4 days post-JCV exposure using ImageJ software (open-source program distributed by the National Institutes of Health) for quantitation of late viral protein (Vp-1) expression determined by intensity of immunoblot banding and represented here as a percentage-based comparison with the control value (no treatment).

Materials and Methods

Progenitor and PDA cell cultures. Human brain-derived progenitor cells (progenitors) from the telencephalon of an 8-week gestational fetal brain were obtained in accordance with NIH guidelines as previously described (20). Progenitor cultures grown at 10 to 40% confluence were at least 98% positive for nestin staining and did not express glial fibrillary acidic protein. Differentiation of progenitors into an astrocytic lineage (progenitor-derived astrocytes [PDA]) was initiated by culture medium substitution as described earlier (21).

Treatments with stimulants/inhibitors of signal transduction pathways on JCV-exposed cultures. Progenitor or PDA cultures were exposed to JCV (Mad-4 variant) at 100 hemagglutination units (HAU)/5 x 10⁵ cells in a minimal covering of appropriate serum-free medium. After overnight JCV exposure, cultures were washed and replenished with appropriate cell-specific growth medium. The PDA were treated with various signal transduction pathway stimulants/inhibitors in a minimum of serum-free Eagle’s medium’s essential medium for 2 h, after which JCV was added for overnight incubation. The JCV medium was then removed, and cultures were replenished with Eagle’s minimum essential medium containing 10% fetal bovine serum, as well as stimulants/inhibitors at concentrations identical to those in the original cultures. For induction of TGF-β1 signaling, culture media were supplemented with 5 ng/ml of recombinant TGF-β1 protein (R & D Systems). All JCV-exposed (controls) and JCV-exposed stimulant/inhibitor-treated cultures were processed 4 days post-JCV exposure as described in “Immunostaining” and “Nuclear extract and whole-cell extract preparation” below.

Immunostaining. Four days after treatments with signal transduction pathway stimulants/inhibitors, cells grown on chamber slides were fixed with 4% paraformaldehyde in phosphate-buffered saline for 20 min at room temperature and permeabilized with 0.1% Triton in phosphate-buffered saline for 10 min at room temperature (RT). To block nonspecific binding of the antibodies used, fixed cells were incubated in TBS-T (Triton-buffered saline [TBS; 25 mM Tris-HCl, pH 7.4, 150 mM NaCl] with 0.1% Tween 20) containing 5% goat serum and 0.1% bovine serum albumin for 2 h at RT. The nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI) to stain nuclei blue. Fluorescent staining was examined using a Zeiss Axiovert microscope, and images were captured with Axiovision software. Comparative counts of total or Vp-1-stained cells were determined using ImageJ software (an open-source program distributed by the National Institutes of Health at http://rsb.info.nih.gov/ij/).

Nuclear extract and whole-cell extract preparation. Four days after treatments with signal transduction pathway stimulants/inhibitors, nuclear fractions and whole-cell extracts were separated as previously described (27). Protein concentrations were determined with the Bio-Rad DC protein assay kit according to the manufacturer’s protocol.

Western blots. Ten to 15 μg of protein from the nuclear extracts or whole-cell lysates above was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4 to 12% Bis-Tris NuPage gels (Invitrogen) and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore). The blotted membranes were blocked with TBS-TM (TBS-T containing 5% nonfat dry milk) for 2 h at RT and probed with appropriate primary antibodies (anti-Vp-1 [developed in our laboratory], anti-simian virus 40 T antigen [OncoGene], anti-MEK1 [Abcam], anti-β-actin [Sigma], anti-Smad2/3, and anti-Smad4 [Cell Signaling]) in TBS-TM. Western blot assays utilizing anti-MAPK antibodies (anti-p44/p42 and anti-phospho-p44/p42 [Cell Signaling]) were performed as described above, with the exception of TBS-TA (TBS-T containing 5% bovine serum albumin) being used throughout in place of TBS-TM. Bound primary antibodies were detected using either an anti-rabbit or an anti-mouse horse- radish peroxidase-conjugated secondary antibody included with the SuperSignal West Pico chemiluminescent substrate kit (Pierce), according to the manufacturer’s protocol.

Transfection experiments. Either 5 μg of a plasmid which codes for constitutively active mutant MEK1 (CA-MEK1) protein (kindly provided by H. Pant, NINDS) or 5 μg of an empty control plasmid vector (pCDNA3; Invitrogen) was transfected into PDA cultures using astrocyte Nucleofector reagent (Amaxa, Inc.). At 16 h posttransfection, the culture medium was replaced with fresh medium containing JCV at 100 HAU/5 x 10⁵ cells. After 8 h of JCV exposure, viral medium was aspirated, cells were washed once, and fresh medium was added. Four days after the transfection (3 days after JCV exposure), whole-cell lysates were prepared for use in Western blot experiments.

Because variants of JCV promoter sequence convey different levels of viral activity, two JCV genomic plasmids, which contain distinct viral promoter sequences, were assayed in comparative immunoblotting experiments for levels of JCV Vp-1 protein expression transcribed and translated into the PDA cultures using astrocyte Nucleofector reagent (Amaxa, Inc.). At 16 h posttransfection, the culture medium was replaced with fresh medium containing JCV at 100 HAU/5 x 10⁵ cells. After 8 h of JCV exposure, viral medium was aspirated, cells were washed once, and fresh medium was added. Four days after the transfection (3 days after JCV exposure), whole-cell lysates were prepared for use in Western blot experiments.

Anchored transcriptional promoter assay (ATPA). The Archetype and Mad-1 JCV promoters were PCR amplified from pJC-CY (32) and pM1TC (8), respectively, with the primers listed below. After amplification, the PCR products were gel purified and ligated into the pATPA vector (29) (the less-activating Archetype, type II singular [II-S]) promoter sequence (12). The DNA nuclear extracts used for these Western blot experiments were prepared 7 days posttransfection.

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inhibitors) containing 10% ACR-NF-1A1.1 and gently rotated overnight at 4°C. After centrifugation to pellet ACR-NF-1A1.1, the supernatants (precleared nuclear extracts) were added to equal volumes of protein binding buffer containing 10% ATP-Arche or ATP-Mad-1. After 2 h at 4°C with gentle rotation, the ATP-Arche and ATP-Mad-1 were pelleted by centrifugation and washed three times with ATPA wash buffer (TBS, pH 7.4, 0.5% Triton X-100, 0.5% glycerol, and protease inhibitors) and once with TBS containing protease inhibitors. Proteins that bound were eluted by boiling the washed ATP-Arche and ATP-Mad-1 for 3 min in Laemmli sulfate-polyacrylamide gel electrophoresis and analyzed by Western blotting using anti-Smad2/3 and anti-Smad4 polyclonal antibodies.

**RESULTS**

To facilitate examination of the molecular factors that affect JCV multiplication in human neural cell types, we previously developed a culture model system based on multipotential human CNS progenitor cells (progenitors) which can be selectively differentiated into either a JCV-susceptible astrocytic (PDA) or a nonsusceptible neuronal (progenitor-derived neurons) lineage (20). These cultures provide nearly pure populations of target cells for infection studies.

**Stimulation of JCV multiplication by TGF-β1 through the MEK signaling pathway.** The activation of MAPK, p38 kinase, JNK, or protein kinase A pathways showed no significant stimulatory effect on JCV multiplication (Table 1). However, the addition of TGF-β1 nearly doubled JCV multiplication (approximately 200% of the control). Addition of the MEK1/2 inhibitor PD98059 or U0126 not only abolished basal viral multiplication but also significantly reduced this TGF-β1-stimulated viral multiplication. To test if these inhibitors negatively affect the binding and entry of JCV, PDA cultures were treated either 4 h before or 4 h after JCV exposure and continuing through to the experimental end point (day 4). For each inhibitor, no difference in Vp-1 expression level was observed between times of viral exposure, suggesting that the effects of these inhibitors on JCV permissiveness are intracellular, not cell membrane associated.

For further confirmation, immunostaining experiments were performed utilizing anti-Vp-1 to assay viral multiplication in JCV-exposed PDA treated with either of the MEK inhibitors PD98059 and U0126, plus both of these conditions with TGF-β1 stimulation (Fig. 1A). The control comparison of PDA not treated with PD98059 or U0126 showed twice as many cells staining for Vp-1 with TGF-β1 stimulation as without TGF-β1 stimulation. Both PD98059 and U0126 blocked nearly all Vp-1 expression in the nonstimulated and TGF-β1-

Cells were fixed, permeabilized, and then stained with anti-Vp-1 (red) to determine relative JCV multiplication. Cellular nuclei were stained with DAPI (blue). (B) Western blot assays, from separate experiments having culture conditions identical to those of the immunostaining, utilized nuclear extracts that were resolved on 4 to 12% gradient gels, transferred to a PVDF membrane, and probed with anti-Vp-1 and anti-β-actin. β-Actin was a consistent immunoblot loading control for nuclear extracts and whole-cell lysates, as determined by comparative experiments with α/β-tubulin antibodies. (C) Comparative counts of total versus Vp-1-stained cells were determined from images with an ×10 magnification using ImageJ software (an open-source program distributed by the National Institutes of Health at http://rsb.info.nih.gov/ij/). Results included are representative of three independent experiments.

**FIG. 1.** (A) Permissive JCV cell type study. Immunostaining of PDA cultures 4 days post-JCV exposure (control) and also with the addition of either 20 μM of PD98059 or 10 μM of U0126. These same three conditions were also tested in the presence of 5 ng/ml of TGF-β1.
stimulated cultures, confirming inhibition of viral multiplication by MEK inhibitors. PDA nuclear extracts analyzed by Western blotting also showed significant inhibition of TGF-β1 stimulation by PD98059 and U0126 (10 μM) found in the literature for MEK1/2 inhibition in similar cell types. Blot assays utilized whole-cell lysates that were resolved on 4 to 12% gradient gels, transferred to a PVDF membrane, and probed with anti-Vp-1 and anti-β-actin. The anti-β-actin blot serves as the total protein loading control. Results included are representative of three independent experiments.

FIG. 2. (A) Western blotting comparison of the effects of various signal transduction pathway stimulators or inhibitors on early viral protein expression in PDA cultures 4 days post-JCV exposure. Blotting assays utilized nuclear extracts that were resolved on 4 to 12% gradient gels, transferred to a PVDF membrane, and probed with anti-T antigen (anti-T) and anti-β-actin. The anti-β-actin blot serves as the total protein loading control. Results included are representative of three independent experiments.

(Fig. 2B). This observation suggests that stimulation of JCV multiplication by TGF-β1 occurs through MEK.

The MEK1/2 inhibitors PD98059 and U0126 effectively reduce JCV multiplication. MEK1/2 are critical members of the MAPK pathway that have been shown to be involved in the growth and survival of cancer cells. The first “nonclassical” kinase inhibitors were the MEK inhibitors PD98059 and U0126. PD98059 is a potent and specific cell-permeable inhibitor of MEK1/2 activation. Likewise, U0126 inhibits MEK1/2 activation but also inhibits the active forms of MEK1/2. Blocking MEK1/2 activity inhibits the phosphorylation-based activation cascade of ERK1/2 and ERK1/2 substrates. Because PD98059 and U0126 inhibition is noncompetitive with respect to ATP and ERK1/2, the specific nature of these MEK1/2 inhibitors was employed to investigate possible connections between MAPK signaling pathways and JCV multiplication. While both PD98059 and U0126 were effective in inhibiting JCV multiplication, as measured by the levels of JCV protein expression detected in immunoblotting experiments (Fig. 3), slightly less viral protein expression was observed with U0126 than with identical concentrations of PD98059.

TGF-β1 increases JCV multiplication in human neural progenitor cells. Progenitor cells support only low-level JCV multiplication compared with that supported by PDA (20). To determine if TGF-β1 stimulates JCV multiplication in nonpermissive cell types, JCV-exposed progenitors were treated with TGF-β1. JCV multiplication was then assessed 4 days post-JCV exposure utilizing anti-Vp-1 for immunostaining of fixed-cell cultures and immunoblotting of whole-cell lysates. Statistical analysis of three individual experiments showed no significant difference in cell numbers between untreated and TGF-β1-treated progenitors, but staining for Vp-1 in the TGF-β1-treated progenitors (15%) was significantly increased above that for untreated cells (0.9%). This stimulatory role of TGF-β1 on JCV multiplication was also confirmed by the immunoblotting results (Fig. 4).

MEK1 plays a role in regulating JCV multiplication. To further examine the role of MEK in JCV tropism, in light of the reduction of viral protein expression by MEK1/2 inhibitors, PDA were transfected with either control plasmid or plasmid encoding constitutively active MEK1 (CA-MEK1) and then exposed to JCV. CA-MEK1-transfected PDA showed enhanced Vp-1 production compared to that of PDA transfected
with control plasmid (Fig. 5). To assess if overexpression of CA-MEK1 affected ERK phosphorylation levels, total ERK1/2 and phosphorylated ERK1/2 levels were measured. Even though ERK1/2 levels were unaffected, phosphorylated ERK1/2 increased with the overexpression of CA-MEK1, demonstrating the functional activity of CA-MEK1.

**TGF-β1 promotes the association of Smads with an active JCV promoter variant.** JCV protein expression is regulated by the association of cellular transcription factors with the viral promoter. Hence, variation of promoter sequence can dictate which transcription factors bind and, thereby, affect viral production. Because of known involvement of Smad proteins in transcriptional regulation and the direct effects of TGF-β1 activation, plays a role in the association of Smad proteins with variants of JCV promoter sequence.

A JCV genomic plasmid with Archetype promoter sequence, which showed low viral protein expression, and a JCV genomic plasmid with Mad-1 promoter sequence, which showed high viral protein expression (Fig. 6A), were used as templates for the amplification of anchored JCV promoter regions. In an approach similar to antibody-based immunoprecipitations, JCV-anchored transcriptional promoters (ATP-Mad-1 and ATP-Arche) were used to test the affinity of DNA binding proteins from nuclear extracts of nontreated, as well as TGF-β1-treated, whole-cell lysates. Nontreated PDA nuclear extracts. Smad proteins that bound neither ATP-Mad-1 nor ATP-Arche, while Smad4 from TGF-β1-treated PDA nuclear extracts. Smad2 was found to associate with TGF-β1, while specific MEK inhibitors decreased it. In cotreatments, MEK inhibitors were the only compounds tested that significantly decreased the TGF-β1 stimulation, identifying MEK as having a central effect on JCV multiplication.

There is evidence that TGF-β1 stimulation of JCV multiplication while specific MEK inhibitors decreased it. In cotreatments, MEK inhibitors were the only compounds tested that significantly decreased the TGF-β1 stimulation, identifying MEK as having a central effect on JCV multiplication.

**DISCUSSION**

In an effort to exploit the cellular signaling pathways used for viral propagation, we screened JCV-exposed PDA cultures via treatments with a number of stimulants and inhibitors of signal transduction pathways. We found that TGF-β1 stimulation of JCV multiplication while specific MEK inhibitors decreased it. In cotreatments, MEK inhibitors were the only compounds tested that significantly decreased the TGF-β1 stimulation, identifying MEK as having a central effect on JCV multiplication.

There is evidence that TGF-β1 levels are increased during immunosuppressive conditions, particularly noteworthy in HIV/AIDS (6). Because PML occurs in the context of JCV reactivation during immunosuppression and is most commonly associated with AIDS, our demonstration of JCV stimulation through a TGF-β1/MEK axis suggests the importance of TGF-β1 in pathogenesis.

It has been previously reported that JCV infection leads to the activation of MAPKs, ERK1/2, within the first few hours of viral exposure (25). However, other MAPK stimulants used in
this study showed no significant effect on JCV multiplication, either alone or in combination with TGF-β1. Also, the comparison of MEK inhibitor treatments, 4 hours before or 4 hours after JCV exposure, revealed that the effects are at the intracellular level, possibly in the nucleus.

The significance of host activation of MAPK in other models of viral infection has been reported. In the HIV-1 model, activation of MAPK is beneficial for viral replication, where inhibition leads to reduced viral replication (11). In addition, involvement of ERK in activity of adenovirus type 7 (1), Borna disease virus (23), influenza A virus (24), and hepatitis C virus (10) suggests a general strategy for enhanced viral replication (17). In our studies here, despite MEK being a specific downstream component of the MAPK pathway and a point of cross talk with other signaling pathways, activation of the MAPK, p38, JNK, or protein kinase A pathway showed no significant increase in JCV multiplication. Even though TGF-β1 alone was sufficient to increase JCV multiplication, this stimulatory effect was significantly diminished by inhibitors that specifically block MEK1/2 activation and/or activity, demonstrating that TGF-β1 stimulation is transduced through MEK. Additionally, the stimulation by overexpression of constitutively active MEK1 points to the central role that MEK activity plays in JCV multiplication.

The site of latency and the molecular events leading to reactivation are critical features of the pathogenesis of CNS diseases associated with latent and/or persistent viruses (18, 22). The transcriptional regulation of JCV expression is the limiting factor governing the range of cell types that can serve as sites of JCV latency and reactivation (18). The understanding that increased TGF-β1 levels occur in the context of immunosuppressive conditions prompted us to test if TGF-β1 addition would increase JCV multiplication in nonsusceptible cell types. Interestingly, TGF-β1 stimulated JCV multiplication in progenitor cells (which normally support only low-level viral multiplication). These results highlight the possibility that even though specific cellular receptors are important for JCV internalization, other factors which act at the level of transcription and replication are crucial for JCV regulation.

A series of reports indicate that several MAPKs can be rapidly activated by TGF-β1 in a manner dependent on cell type and condition (19). The biochemical link between the TGF-β1 and MAPK pathways has been elusive due to the paucity of supportive evidence. Smad proteins are the only known TGF-β1 receptor substrates capable of signal transduction. TGF-β1 conveys a signal by translocating Smads into the nucleus. Translocated Smads are then regulated in the ability to affect gene expression by forming complexes with DNA binding cofactors and transcriptional coactivators/corepressors.

The level of JCV multiplication depends upon the nucleotide sequence of the viral regulatory region and the interaction of this promoter sequence with host cell transcription factors (18, 26, 28). Nucleotide sequences that act as transcriptional promoters are located between the early and late protein coding regions and contain the origin of DNA replication (8, 18). These sequences also contain TATA boxes, as well as binding sites for Sp1, YB1, sup2, pur-α, c-Jun, and NF-1. The increased viral multiplication in PDA stimulated by TGF-β1 treatment revealed concurrent increases of Smad2/4 association with JCV-Mad-1 promoter sequence (a highly active JCV regulatory region variant), compared to JCV Archetype promoter sequence (a much less active regulatory region variant). Because TGF-β1 is involved in activating Smads, which can translocate to the nucleus, Smads may associate with highly active JCV promoter sequences as transcriptional coactivators of JCV gene expression. Our findings support the notion that binding of Smads to highly active JCV promoters is, in part, responsible for fostering JCV propagation and therefore suggest the role that TGF-β1 plays in stimulating JCV multiplication. In line with other published reports (19), our results raise the possibility that TGF-β1 may simultaneously activate Smad and MAPK pathways which then physically converge on the target, in this case the JCV promoter.

Currently, we are studying Smad cofactor binding to JCV promoters and examining if Smad association requires MEK activity. We are also testing MEK1/2 inhibition on the activity
of a closely related polyomavirus, BK virus, which is also activated during immunosuppressive conditions.

In conclusion, we have demonstrated that a TGF-β1/MEK axis is involved in JCV multiplication. We have shown that TGF-β1 promotes association of Smads with a highly active variant of JCV promoter sequence and that JCV multiplication in human glial cultures can be significantly reduced by the administration of MEK1/2 inhibitors. Therefore, greater understanding of the MEK1/2 signaling pathway may lead to novel antiviral therapies.

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