Transcriptional Activation of JC Virus by Human T-lymphotropic Virus Type I Tax Protein in Human Neuronal Cell Lines*

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Polyomavirus JC (JCV) causes the human demyelinating disease, progressive multifocal leukoencephalopathy (PML). The recent demonstration of cases of PML in association with human T-lymphotropic virus type I (HTLV-I) infection prompted us to examine whether the HTLV-I-encoded regulatory protein Tax activates JCV transcription. By employing a dual luciferase assay, we initially found that the expression of Tax activated the transcriptional potential of both early and late promoters of JCV in human neuronal but not in non-neuronal cells. We subsequently analyzed the mechanism of Tax-induced activation of the JCV promoter in neuronal cells with the following results: 1) the JCV promoter that lacks the NF-kB-binding motif could not be activated by Tax; 2) the overexpression of IxBa abolished Tax-induced transcriptional activation of the JCV promoter; and 3) a Tax mutant (M22) lacking the potential for activation via the NF-kB pathway did not activate the JCV promoter. Furthermore, Tax enhances the gene expression of JCV T antigen and VP1. We examined mechanisms of the cell-specific activation of the JCV promoter by Tax. Electrophoretic mobility shift assay demonstrated the presence of Tax-bound protein(s) that were specifically present in non-neuronal cells. This study is the first demonstration of the activation of JCV promoter by HTLV-I Tax in an NF-kB-dependent manner.

JC virus (JCV)1 is known to be a causative agent of the human demyelinating disease, progressive multifocal leukoencephalopathy (PML), that is observed mainly in immunosuppressive states such as in AIDS, advanced stage of malignant tumors, or following organ transplantation (1). JCV belongs to the polyomaviridae of double-stranded DNA viruses that also include simian virus 40 (SV40) and BK virus. Although the complete genome of JCV shares extensive homology with SV40 and BK virus, the nucleotide sequences of the transcriptional regulatory region of JCV are quite different. In addition, the JCV promoter sequence can be classified into two types, non-virulent type (archetype) and pathogenic type (PML type). The regulatory region of JCV isolated from PML brain (PML type JCV) is generally composed of 98-base pair (bp) tandem repeats including TATA sequences, whereas archetype JCV that is excreted in the urine in asymptomatic individuals has only one copy of the 98-bp sequence flanked with additional 23- and 66-bp residues. Therefore, it has been postulated that the rearrangement of the regulatory region of JCV from archetype sequence to that of 98-bp tandem repeats permits cytopathic proliferation of JCV in glial cells that results in PML (2). A number of transcriptional factors, such as NF-1, NF-kB, YB-1, Pur, Tst-1, GF-1, and T antigen have been shown to bind JCV regulatory regions in in vitro analysis (3) and are thought to be involved in the species and tissue specificity of JCV propagation. Human immunodeficiency virus (HIV)-encoded Tat protein has also been reported to transactivate the JCV promoter (4). Despite the extensive efforts to clarify the transcriptional factors involved in the JCV activation, the precise mechanisms of its regulation in central nervous tissue and of pathogenesis of PML are still under the investigation.

Recently, we and others (5, 6) have described cases of PML in the setting of human T-lymphotropic virus type I (HTLV-I) infection. These have raised the question of whether HTLV-I proteins could have been involved in the transcriptional activation of the JCV promoter. HTLV-I regulatory protein Tax is a 40-kDa protein that is known to activate several cellular and viral genes including HIV, cytomegalovirus (CMV), and SV40. In this report, we demonstrate that expression of Tax activates the transcriptional potential of JCV promoter in human neuronal/glial cells, whereas transactivation of the virus promoter by Tax was not detected in non-neuronal cells.

We could also demonstrate the following: 1) JCV that lacks NF-kB motif in its regulatory region cannot be activated even in the presence of Tax; 2) overexpression of the inhibitory protein, IxBa, abolishes Tax-induced transcriptional activation of JCV promoter; and 3) a Tax mutant (M22) lacking the potential for NF-kB activation fails to transactivate the JCV promoter. Thus three independent experimental approaches demonstrate that Tax transactivation of the virus promoter occurs via the NF-kB pathway. Furthermore, the detection of a protein com-
plex bound by JCV NF-κB oligonucleotides using EMSA has suggested there is an inhibitory protein function in non-neuronal cells that suppresses JCV promoter activity. These results provide new insights into the understanding of the activation of JCV and a possible role for HTLV-I infection in the pathogenesis of certain cases of PML.

**Experimental Procedures**

**Cells and Tissue Culture**—Human neuroblastoma cell line IMR-32 (JCRB 9050), human embryonic kidney cell line HEK293 (JCRB 9068), human cervical carcinoma cell line HeLa (JCRB 9004), human malignant melanoma cell line MeWo (JCRB 0066), and human mixed glioma cell line KG-1-C (JCRB 0236) were provided from the Health Science Research Resources Bank (Japan). Human glioblastoma cell lines U-373 MG (ATCC HTB 17), U-87 MG (ATCC HTB14), and U-138 MG (ATCC HTB16) were purchased from the American Type Culture Collection. All cell lines were maintained in Dulbecco’s minimal essential medium supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin) from Sigma. None of the cell lines used these experiments expressed T antigen which is known to activate JCV promoter (data not shown).

**Plasmids**—Tet-1-Oct16 cDNA was kindly provided from Dr. Wegner (7) and subcloned into the mammalian expression vector, pFLAG-CMV-2 (Sigma) named pFLAG-Tat1. The pC1–4–pJCV plasmid (HSRRB YG015), the source for the PMT type JCV regulatory regions, was provided from Health Science Research Resources Bank. The luciferase reporter vector, pGL3-Basic, and internal control vector, pRL-TK, were purchased from Promega. The IκBα expression vector (pIκBα) was kindly provided from Dr. Peyron through Dr. Ouchi (8). As the JCV regulatory regions are known to be quite polymorphic, we chose the regulatory regions of both CY (9) and Mad1 (10) as archetype and PML promoters. For construction of Tax mutant (M222), two amino acids, Thr130 and Leu131 of the wild-type Tax were substituted by Ala130 and Ser131 using oligonucleotide site-directed mutagenesis. Both the wild-type Tax and M222 fragments were separately subcloned into the mammalian expression vector, pCXX, PLAG, generating N terminus FLAG epitope (DYKDDDDK)-tagged fusion protein (12) and were named pFLAG-TaxWT and pFLAG-TaxM22, respectively.

**Transfection and Luciferase Assays**—Transient transfections were carried out by the Effectene method (Qiagen) following the manufacturer’s instruction. In some experiments 1.0 × 10^5 cells were plated onto 12-well plates, and for other experiments 3–10 × 10^5 cells were seeded onto 60-mm plates and grown overnight. Vectors, including the reporter vectors, the internal Renilla luciferase control vector (pRL-TK), and other protein expression vectors were co-transfected as indicated in the figure legends. The total amount of transfected DNA for each transfection was standardized. The 60-mm plate was up to 1 μg. All assays for firefly and Renilla luciferase activity were performed using one reaction tube sequentially (Promega). Briefly, at 48 h post-transfection, the cells were washed with phosphate-buffered saline and lysed with Passive Lysis Buffer. After a freeze/thaw cycle, samples were mixed with Luciferase Assay Reagent II, and the firefly luminescence was measured with a Luminometer (Turner Designs, CA). Next, samples were mixed with the Stop & Glo reagent, and the Renilla luciferase activity was measured as an internal control. Finally, luciferase activity was calculated as follows: (firefly luciferase activity of the sample/Renilla luciferase activity of the sample) — (firefly luciferase activity of the background (control vector)/Renilla luciferase activity of the background (control vector)). The results are confirmed by three independent transfections. The data are presented as mean values ± S.D. Comparisons between groups were made with the t test for paired observations.

**Immunoblotting**—Anti-FLAG monaclonal antibody (M2) was purchased from Sigma, and anti-IκBα (C-21), anti-p50, and anti-p65 (sc-9001) were purchased from Santa Cruz Biotechnology. Cell lysates from each experiment were resolved by 11% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto nitrocellulose filters, and immunoblotted with the antibody. Immunoreactive bands were detected with an anti-Ig conjugated with horseradish peroxidase followed by ECL (Amersham Pharmacia Biotech) and analyzed with the LAS-1000 plus (Fuji Film, Japan).

**Reverse Transcription (RT)-PCR Analysis of Viral Proteins**—The closed circular CY DNA was generated from the plasmids, pJCV-CY, after EcoRI digestion and self-ligation. This closed circular viral DNA was co-transfected with Tax expression vector, pFLAG-TaxWT, into the KG-1-C cells plated onto 6-well plates the day before transfection. At two days post-transfection, the serum concentration of conditioned media was decreased from 10 to 2%. Five days after transfection, cells were harvested and used for RT-PCR of mRNAs of the viral early and late proteins, T antigen and VPI, respectively, and the Tax protein. Total RNA was isolated with TRIzol reagent (Life Technologies, Inc.), and 1 μg of total RNA was reverse-transcribed using Superscript II (Life Technologies, Inc.) according to the instructions of manufacturer. PCR was performed using AmpliTaq Gold DNA polymerase (Perkin-Elmer). Primers were as follows: TtAgF (5′-ATG GAA AAA GTC CTA AGG G-3′) and TtAgR (5′-TTA AAG CTT TAG ATC CCT GTA GG-3′) were used to amplify the JCV early protein, T antigen (nucleotides 5013-5029).
FIG. 2. Activity of the JCV promoters in the presence of Tax. A, influence of Tax on the JCV promoter activities in various cell lines. Transient transfections were carried out in IMR-32, KG-1-C, HEK293, U-87 MG, and U-138 MG cell. The following plasmids were used for the experiments: pJCV-MadK or l-luc, pJCV-Cyc or l-luc, pFLAG-TaxwT, pCXN2-FLAG, and pRL-TK. Each reporter vector pJCV-MadK or l-luc or pJCV-Cyc or l-luc was co-transfected with either mock vector, pCXN2-FLAG, or Tax expression vector, pFLAG-TaxwT, into the five different human cell lines. Luciferase activities in extracts from transfected cells were determined in at least three independent experiments. The values of each JCV promoter reporter vectors were calculated by dividing the amount of luciferase activity of the pJCV-Cyc-luc in the absence of Tax in each cell line. The activity of the pJCV-Cyc-luc in the absence of Tax is therefore 1 in all cell lines tested. Data are presented as relative luciferase activity ± S.D. The fold activation (indicated below) was calculated for each reporter plasmid by comparing values from cells transfected with pFLAG-TaxwT with values from cells transfected with mock vector pCXN2-FLAG. Comparisons between two groups were made with the t test for paired observations. *, p < 0.05, and **, p < 0.01 statistically difference from cells in the absence of Tax. B, upper panel, various amounts (indicated below) of the pFLAG-TaxwT plasmid were introduced into both HEK293 cells and IMR-32 cells co-transfected with 60 ng of pJCV-Cyc-luc, 3 ng of pRL-TK vector and mock plasmid to adjust the total amounts of transfected DNA to 300 ng. At 48 h post-transfection, extracts were prepared, and both firefly and Renilla luciferase intensities were determined. Luciferase activities in extracts from transfected cells were determined in at least three independent experiments. Data are presented as mean fold activation ± S.D., which were calculated for each reporter plasmid by comparing values from cells transfected with pFLAG-TaxwT with values from cells transfected with mock vector pCXN2-FLAG. Lower panel, the expression levels of FLAG-Tax fusion protein were estimated by the Western blotting with the anti-FLAG antibody. Solid arrows indicate the signals of FLAG-Tax protein.

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to 4495); VP1-F (5'-ATG GCC CCA ACA AAA AGA AAA GG-3') and VP1-R (5'-CAC TGT GGC ATT CTT TGG A-3') were used to amplify the JCV late protein, VP1 (nucleotides 1469 to 1999); Tax-566F (5'-AGC GAA TAG AAG AAC TCC TC-3') and Tax-R (5'-TCA GAC TTC TGT TTC CGA-3') were used to amplify HTLV-I Tax (nucleotides 566 to 1062). The PCR conditions were as follows: for JCV proteins, 95 °C for 10 min; 3 cycles of 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min; 27, 37, or 47 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, and final extension of 72 °C for 10 min, for Tax protein, 95 °C for 10 min; 3 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min; 42 cycles of 94 °C for 1 min, 62 °C for 1 min, 72 °C for 1 min, and final extension of 72 °C for 10 min. Amplified DNAs were run on 1.2% agarose gel and quantified by Imaging Densitometer (Bio-Rad).

Immunoprecipitation and Western Blot Analysis—Tax- and/or Tst-1-
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Established that Tax activates NF-κB and reduces its transcriptional activity that is not rescued by Tax—

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared from HEK293 cells, Tax-transfected HEK293 cells, IMR-32 cells, and Tax-transfected IMR-32 cells employing Dignam’s methods (14) with slight modifications. Binding reactions were carried out as follows: 10⁷ cpm of 32P-labeled oligonucleotide probe, 0.5 μg of poly(dI-dC)poly(dI-dC), and 0.5 μg of bovine serum albumin in a total volume of 25 μl. Following a 30-min incubation on ice, the resulting complexes were resolved on a 5% polyacrylamide gel containing 0.25× Tris borate EDTA (TBE) buffer at 4 °C. For supershift band assays, samples were incubated with anti-p50, anti-p65 antisera (sc-1190X and sc-109X, respectively), and anti-κB antibody on ice for 1 h, bound to 15 μl of protein G-Sepharose beads (Amersham Pharmacia Biotech). The bound proteins were eluted with SDS-sample buffer from the beads after washing with ice-cold lysis buffer. Western blot analysis was carried out with a rabbit anti-FLAG polyclonal antibody (Zymed Laboratories Inc.).

Results

Activation of the JCV Promoter by Tax in Human Neuronal Cells—To evaluate the transcriptional activity of the JCV promoter in the presence of Tax, each of the luciferase reporter vectors with the JCV promoter derived from archetypal type CY or PML-type Mad1 strain were co-transfected with the Tax expression vector, pFLAG-TaxWT, into five different human cell lines. As the regulatory domains of JCV are comprised of two independent promoters, which drive the viral early and late proteins of both archetype and PML type, we examined the transcriptional activity of all four promoters: CY early, CY late, Mad1 early, and Mad1 late. As shown in Fig. 2A, we found that Tax could activate the JCV promoter in the neuronal cells, including neuroblastoma cells, IMR-32 cells, and neuroglial cells, KG-1-C, U-87 MG, and U-138 MG. In particular, in IMR-32 and KG-1-C cells, the activity of the JCV promoters was significantly increased with 2.46–95.60-fold activation by Tax. In contrast, luciferase activity was not enhanced by Tax in the human embryonic kidney HEK293 cells. In addition, we have examined other non-glial cell lines, HeLa and MeWo, and have found that the basal transcriptional activities of JCV in these cell lines was from 1/100 to 1/100 less than those of HEK293 cells, and the activities were not significantly changed even in the presence of Tax (data not shown).

To confirm that Tax did not activate the JCV promoters in HEK293 cells, we carefully compared the correlation of Tax protein expression level and transcriptional activity in both HEK293 and IMR-32 cells, and we found that significant expression of Tax failed to activate the CY promoter in HEK293 cells (Fig. 2B). These results suggest that in the neuronal cell lines, Tax significantly activates all four JCV promoters used in this experiment CY early, CY late, Mad1 early, and Mad1 late.

Deletion of the NF-κB Motif in the JCV Promoter Reduces Its Transcriptional Activity That Is Not Rescued by Tax—It is well established that Tax activates NF-κB and reduces its transcriptional activity that is not rescued by Tax. Since both the Mad1 and CY type of JCV also have the NF-κB motif in the 5′ region upstream of the 98-bp box in their regulatory regions (Fig. 1), we hypothesized that Tax activated the JCV promoter via the NF-κB-binding motif. We constructed JCV promoter-driven reporter plasmids that lack NF-κB-binding motif, pJCV-MadΔκB5′-luc, or pJCV-CYΔκB5′-luc, and these were co-transfected into U-87 MG cells with the Tax expression vector, pFLAG-TaxWT. As shown in Fig. 3A, in the absence of Tax the activities of JCV promoters, CY early, CY late, Mad1 early, and Mad1 late, were greatly reduced by deletion of the NF-κB motif, and in particular, the activity of the CY late promoter was completely abrogated. Moreover, overexpression of Tax did not rescue the reduced transcriptional activities of these mutated JCV promoters lacking the NF-κB motif. In these experiments, the expression levels of Tax were confirmed by Western blotting. Representative data are shown in Fig. 3B. These results indicate that the NF-κB motif is necessary for Tax-induced transactivation of the JCV promoter and also for the basal level of transcription of the JCV promoters with the exception of the CY early promoter.

Deletion of the NF-1 Motif of the JCV Promoter Does Not Diminish Tax-induced Activation of Transcription—To confirm the specificity of the NF-κB motif in Tax activation, we also prepared a deletion mutant of the NF-1 sequence that has been shown to be important in the JCV regulatory region. When the NF-1 deletion mutant reporter vector pJCV-MadΔ1, −1-luc was co-transfected with Tax expression vector pFLAG-TaxWT into U-87 MG cells, the transactivation of the NF-1 deletion mutant promoter was similarly enhanced to that of the wild type (Fig. 3A). These results suggest that the NF-1 motif is not necessary for Tax-induced transactivation and also not necessary for basal level transcription of the JCV promoter in neuroglial cells.

Overexpression of IκBα Inhibits Tax-induced Activation of the JCV Promoter—Recent reports have demonstrated that the overexpression of IκBα provides a dominant negative effect on the NF-κB pathway diminishing the signals from IKK that results in the retention of NF-κB in the cytoplasm (15). We investigated whether overexpression of IκBα could inhibit the

Fig. 3. Influence of NF-κB motif deletion mutant on the JCV promoter activities activated by Tax. A, transient transfections were carried out in U-87 MG cells. Each of wild-type reporter vectors pJCV-Mad5′-luc, pJCV-CY5′-luc, or NF-κB motif deletion reporter vectors pJCV-MadΔκB5′-luc, or pJCV-CYΔκB5′-luc was co-transfected into U-87 MG cells with either pCXN2-FLAG or pFLAG-TaxWT. Data are presented as mean luciferase activity ± S.D. *p < 0.05, and **p < 0.02 statistically difference between two groups. B, the expression of FLAG-Tax fusion protein. Representative results are shown. The transfected plasmids in each cell culture were indicated over the figure. The position of the FLAG-Tax fusion protein is indicated with a solid arrow.
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Transactivation of the JCV promoters by Tax. The IκBα expression vector (pIkBα) was co-transfected with Tax expression vector, pFLAG-TaxWT, and each of the luciferase reporter vectors derived from either CY or Mad1-strain into U-87 MG cells. As shown in Fig. 5A, overexpression of IκBα completely inhibited the enhancement of transcriptional activity of the JCV promoters by Tax. In these experiments, the protein expression levels of both the Tax with FLAG tag and IκBα were confirmed by Western blotting using the anti-FLAG antibody and anti-IκBα antibody, respectively. Representative data are shown in Fig. 5B.

The M22 Tax Mutant Does Not Activate the JCV Promoter—The M22 Tax mutant (T130A and L131S) has been shown to lack the ability to activate the NF-κB-dependent promoters (16). For further confirmation that Tax activates the JCV promoter through the NF-κB pathway, the M22 Tax mutant was also examined for its ability to transactivate the JCV promoter. The wild-type reporter vector, pJCV-CY-Eo-rLuc, was co-transfected into U-87 MG cells with either pCXN2-FLAG or pFLAG-TaxWT. Data are presented as mean luciferase activity ± S.D. B, the expression of FLAG-Tax fusion protein. The transfected plasmids in each cell culture were indicated above the figure. The position of the FLAG-Tax fusion protein is indicated with a solid arrow.

Enhancement of Gene Expression of JCV Early and Late Proteins by Tax.—We investigated if Tax enhances the transactivation of the JCV promoters by Tax. We initially examined the expression levels of the NF-κB subunits, p50 and p65, in HEK293 and IMR-32 cells. As shown in Fig. 7, Tax significantly enhanced the p50 expression in HEK293 cells compared with that in IMR-32 cells, whereas there was a similar expression levels of p65 by Tax in both cell lines. Thus, the significant increase in p50 expression levels induced by Tax in HEK293 cells might be involved in the suppression of Tax.
transactivation of the JCV promoter.

Next, we employed an EMSA to analyze JCV NF-κB motif-binding proteins in HEK293 and IMR-32 cells. The EMSA demonstrated that in nuclear extracts from both IMR-32 and HEK293 cells Tax enhanced a major band (A, Fig. 9B). Furthermore, it could be demonstrated that an additional Tax enhanced band B exhibiting a lower mobility than band A could be detected only in HEK293 cells (Fig. 9B). The elimination of these bands by a cold probe containing the JCV NF-κB motif in a dose-dependent manner demonstrates the specific interaction of the proteins with the probe. To examine the components of these protein complexes, the supershift assay was performed using anti-Tax, -p50, and -p65 antibodies. For both cell lines, the anti-Tax antibody supershifted the band A, and for HEK293 cells, the anti-p50, -p65, and -Tax eliminated band B. No effect was observed on band A in the supershift assay with anti-p50 or p65 antibodies, and this is presumably due to the fact that their potential binding sites are blocked by Tax (19).

**DISCUSSION**

It has been clearly demonstrated that JCV infection is increasingly important in HIV-1-infected individuals, and the incidence of PML has risen dramatically with the epidemic of AIDS. Evidence for a direct role for HIV in JCV activation has been provided by studies showing transactivation of the JCV late promoter by the HIV-1 Tat protein (4). Recently, we documented two autopsy-proven cases of PML without immunosuppression in the setting of co-infection of another retrovirus HTLV-I, and this raised the question of whether HTLV-I could activate JCV in a similar manner as HIV-1. We investigated if HTLV-I Tax could play a pivotal role in JCV activation, as Tax has been shown to alter the expression levels of a large number of cellular genes (20), and it has been reported that Tax can transactivate the promoters of several viral genes, including those of HIV, SV40, and CMV (21–23).

In our studies we could demonstrate that in neuronal/glial cells, the transcriptional activities of the JCV promoters were significantly enhanced by Tax. Furthermore, it could be shown...
that Tax could not transactivate mutants of the JCV promoter lacking the NF-κB motif. In contrast, Tax could enhance the promoter activity of another mutant of JCV promoter with a deletion of the NF-1 motif sequence. In addition, we have shown that overexpression of IκBα dramatically inhibited the enhancement of JCV promoter induced by Tax and that the M22 Tax mutant that abrogates the ability of the protein to activate via NF-κB could not transactivate the JCV promoters. Taken together, we have demonstrated that HTLV-I Tax activates the JCV promoters, CY early, CY late, Mad1 early, and Mad1 late, via the NF-κB-dependent pathway. We also investigated whether Tax could enhance the expression of JCV proteins. In this experiment, both CY and Mad1 DNAs were co-transfected with the Tax expression vector into KG-1-C cells. In contrast to the CY-transfected cells, in which the gene expression was markedly enhanced by Tax, over 70% of the Mad1-transfected cells showed cytoplasmic vacuolating change leading to cell death from the 2nd day after transfection in the presence of Tax (data not shown). As such it was impossible to detect the mRNAs of the JCV proteins in Mad1-transfected cells. It is well known that JCV has a highly restricted cell type specificity and that transactivation of JCV by HTLV-I Tax occurs only in neuronal/glial cells. To analyze the mechanism of the neuronal specificity, we examined whether Tax could transactivate the JCV promoter in the setting of overexpression of the brain-specific transcriptional factor, Tst-1/Oct6. We could demonstrate using immunoprecipitation assays that Tax bound Tst-1/Oct6 in vivo. Because Tst-1/Oct6 is known to combine with a DNA motif in the vicinity of the TATA box (24), it was hypothesized that Tax might form a complex with NF-κB, Tst-1/Oct6, and the TATA-binding protein and activate the JCV promoter. However, despite overexpression of Tst-1/Oct6 in HEK293 cells Tax failed to transactivate the JCV promoter.

We have also examined the expression levels of the NF-κB family, p50 and p65 in the presence of Tax, because p50 inhibits whereas p65 activates the JCV promoter via the NF-κB motif (18). In the presence of Tax, there was a significant increase of p50 expression levels in nuclear extracts from HEK293 cells compared with IMR-32 cells, whereas p65 was equally expressed in both cell lines. This result suggests the possibility that a surplus of p50 induced by Tax might be involved in suppression of the transactivation of JCV in HEK293 cells.

Next, we have carried out preliminary studies to identify cellular factors that might be involved in the neuronal cell-specific transactivation of JCV using EMSAs. In nuclear extracts from HEK293 cells, but not IMR-32 cells, a distinct band...
(B) was detected with the JCV NF-κB probe. A second band (A) was recognized in both cell types. The latter band (A) probably represents a complex of Tax and NF-κB, because this band was shifted by anti-Tax antibody and Tax could interact with both p65 and p50 in immunoprecipitation assays (data not shown). These findings raise the possibility that HEK293 cell-specific protein(s) function suppressively in Tax-induced activation of the JCV promoter. The narrow tissue-tropism of JCV is thought to be dependent on its regulatory region which a number of transcriptional factors bind, and most of these proteins function as up-regulators of the JCV promoter. In this study, we have shown the neuronal/glial cell-specific transactivation of the JCV promoter by HTLV-I Tax, and we demonstrate the existence of a non-neuronal cell-specific protein complex, which interacts with NF-κB and which may be associated with the inhibition of the transactivation activity of Tax. Ongoing studies should identify the components of the latter and of the neuronal-specific factors involved in transactivation.

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