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Human Immunodeficiency Virus-Restricted Replication in Astrocytes and the Ability of Gamma Interferon To Modulate This Restriction Are Regulated by a Downstream Effector of the Wnt Signaling Pathway▽

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Astrocyte dysregulation correlates with the severity and the rate of human immunodeficiency virus (HIV)-associated dementia (HAD) progression, highlighting a pivotal role for astrocytes in HIV neuropathogenesis. Yet, astrocytes limit HIV, indicating that they posses an intrinsic molecular mechanism to restrict HIV replication. We previously established that this restriction can be partly overcome by priming astrocytes with gamma interferon (IFN-γ), which is elevated in the cerebral spinal fluid of HAD patients. We evaluated the mechanism of restrictive HIV replication in astrocytes and how IFN-γ priming modulates this restriction. We demonstrate that the downstream effector of Wnt signaling, T-cell factor 4 (TCF-4), is part of a transcriptional complex that is immunoprecipitated with HIV TAR-containing region in untreated astrocytes but not in IFN-γ-treated cells. Blocking TCF-4 activity with a dominant-negative mutant enhanced HIV replication by threefold in both the astrocytoma cell line U87MG and primary fetal astrocytes. Using a TCF-4 reporter plasmid, we directly demonstrate that Wnt signaling is active in human astrocytes and is markedly reduced by IFN-γ treatment. Collectively, these data implicate TCF-4 in repressing HIV replication and the ability of IFN-γ to regulate this restriction by inhibiting TCF-4. Given that TCF-4 is the downstream effector of Wnt signaling, harnessing Wnt signaling as an intrinsic molecular mechanism to limit HIV replication may emerge as a powerful tool to regulate HIV replication within and outside of the brain.

Although the predominant cell target for productive human immunodeficiency virus (HIV) replication is activated CD4+ T cells, HIV can enter other cells with limited productive HIV replication (32). Understanding the innate molecular mechanism(s) that restricts HIV replication in these cells can be a powerful therapeutic approach, if translated to more permissive targets of HIV replication. One such cell type that efficiently restricts HIV replication is the astrocyte (23).

Astrocytes constitute 40 to 70% of cells in the human brain. They play pivotal homeostatic and regulatory functions in maintaining the integrity of the blood-brain barrier and the survival of neurons (9, 47). HIV DNA and particles are detected within astrocytes of postmortem brain of adult HIV-positive patients (18, 51, 54), albeit at a lower frequency than microglia and infiltrating lymphocytes. HIV in vitro infection of astrocytes is restricted with production of few viral progeny (23). This restriction is unique because it leads to an initial burst of low-level HIV, followed by the accumulation of multiply spliced mRNAs (Tat, Rev, and Nef) with the translation of these proteins on occasion but without completing the viral life cycle (19, 36, 44, 50). Productive infection can be reestablished following stimulation with tumor necrosis factor alpha and interleukin-1β (1, 3, 10, 33, 42), but HIV levels still consistently remain far lower than those documented in more HIV-permissive cells, such as microglia, CD4+ T cells, and monocytes. Limited HIV replication in primary astrocytes and astrocytoma cell lines is a consequence of blocks at multiple steps in the viral life cycle, including blocks at entry and postentry events. Bypassing entry requirements through transfection of full-length HIV provirus plasmid led to a transient burst of HIV replication followed by a rapid nonproductive HIV stage (4a, 22, 50, 50a), which represents a single round of HIV replication and indicates that restriction at entry level is a major blocking step. Yet when the level of HIV replication was normalized to transfection efficiency, the level of HIV in astrocytes was 50-fold lower than that in transfected HeLa cells (22, 23). Further, HIV pseudotyped with envelope glycoproteins of amphotropic murine leukemia virus or vesicular stomatitis virus (VSV) demonstrated significant productive viral replication (5, 43a). Replication of the VSV-pseudotyped HIV is further enhanced when an intracellular inhibitor of HIV replication is blocked (23). These data suggest that while block in entry is a major step in restricted HIV in astrocytes, other intracellular events may contribute to this restriction.

The mechanism(s) of intracellular restriction of HIV replication in astrocytes is still not clearly delineated. T-cell factor 4 (TCF-4) was demonstrated to be a potent repressor of HIV replication in astrocytes (55). TCF-4 inhibited both basal and Tat-mediated transactivation of the HIV long terminal repeat

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(LTR) (55). The mechanism of TCF-4 regulation of HIV replication is also not clearly defined. TCF-4 was shown to form a complex with Tat, presumably inhibiting Tat binding to transactivation response (TAR). More recently, TCF-4 was shown to interfere with Sp1 transcription factor, leading to modulation of its capability to activate the HIV LTR by binding to its cognate sites (40).

TCF-4 is a downstream effector of the Wnt pathway. Wnt genes encode a large family of soluble secreted glycoproteins that are differentially expressed and regulate neurogenesis of the developing brain (17) and T-lymphocyte development (46, 48). The canonical/β-catenin-dependent pathway of Wnt signaling is initiated by binding of Wnt proteins to one of the eight members of the Frizzled receptor family. This signal transduction ultimately leads to stabilization of β-catenin, because it is not phosphorylated by the serine/threonine kinase (glycogen synthase kinase 3). Active β-catenin binds the lymphoid enhancer binding factor (LEF)/TCF family of transcription factors (LEF-1, TCF-1, TCF-3, and TCF-4), displacing their repressors such as transducin-like enhancer in humans and Groucho in *Drosophila melanogaster*, and this TCF–β-catenin complex translocates to the nucleus, where it binds to TCF/LEF cognate DNA sequences, regulating gene transcription. TCF–β-catenin target genes include c-*myc*, cyclin D, TCF-4, LEF-1, c-Jun, and CD44, among others. In the absence of a Wnt signal, defined by the lack of Wnt protein binding to Frizzled, β-catenin is phosphorylated and associates with a protein complex (glycogen synthase kinase, axin, and the tumor suppressor protein adenomatous polyposis coli) that tags it for degradation. Without active β-catenin in the nucleus, TCF and LEF remain associated with their repressors on their *cis* elements and inhibit gene transcription.

We previously demonstrated that prestimulation of astrocytes with gamma interferon (IFN-γ) can overcome their restriction of HIV replication in both an astrocytoma cell line (U87MG) and primary human fetal astrocytes (HFA). Because studies linking TCF-4 to HIV repression in astrocytes were based on cotransfection of TCF-4 and Tat, it was not clear if TCF-4 is intrinsically expressed in astrocytes. We evaluated the role of TCF-4 in regulating HIV replication and in mediating the effect of IFN-γ on regulating HIV productive replication in astrocytes. These studies are critical to devise strategies to harness these key signaling events to limit HIV replication within and outside of the brain.

MATERIALS AND METHODS

Isolation of HFA and cell culture. The astroglioma cell line U87MG was obtained from the NIH AIDS Research and Reference Reagents Program (Germantown, MD). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO) and 2% penicillin-streptomycin (Gibco Invitrogen) and used in these experiments at approximately 80% confluence. HFA were purified from second-trimester aborted fetuses. First, mechanically dissociated fetal brain tissue (Advanced Bioscience Resources, Alameda, CA) was cultured in Dulbecco's modified Eagle's medium (Gibco Invitrogen) supplemented with 10% fetal bovine serum, 2% penicillin-streptomycin, and amphotericin B (Fungizone; Gibco Invitrogen). At each passage, the cells were incubated with 1× trypsin-EDTA (Gibco Invitrogen) for 5 min at 37°C, and the microglia, which remained attached, were discarded while the detached cells, astrocytes, were used for the next passage, cells from which were cultured for 2 weeks. This was repeated for three passages until the cultures were greater than 95% positive for glial fibrillary acidic protein, an astrocyte-specific marker, and less than 2% positive for CD68, a microglial marker, as measured by flow cytometry.

IFN-γ treatment and HIV infection. Astrocytes were treated with 100 ng/ml of IFN-γ (BD Pharmingen, San Jose, CA) or left untreated for 24 h followed by HIV infection and maintenance of IFN-γ postinfection. For infection experiments, astrocytes at 70 to 80% confluence were incubated with HIV_{BAL} (NIH AIDS Research and Reference Reagents Program, Germantown, MD) at 10 ng p24/1 × 10^6 cells for 24 h and then washed three times. In some experiments, cells were trypsinized after HIV infection to further ensure removal of bound virus prior to culturing. HIV infection was monitored at day 7 postinfection by measuring p24 levels by conventional enzyme-linked immunosorbent assay (ELISA) (National Cancer Institute, Frederick, MD).

Immunofluorescence staining and flow cytometric analysis. To assess the level of purity of HFA cultures, 1 × 10^6 cells were detached with EDTA and incubated with 5% human serum and 1% bovine serum albumin for 30 min at room temperature. Cells were washed, permeabilized, fixed according to a standard protocol (Caltag, Burlingame, CA), and stained with mouse anti-glial fibrillary acidic protein (BD Pharmingen) conjugated to allophycocyanin or fluorescein isothiocyanate, mouse anti-CD68–phycoerythrin, mouse anti-microtubule-associated protein 2–allophycocyanin, and mouse antinestin-fluorescein isothiocyanate antibodies (BD Pharmingen). Fluorescence was evaluated with a FACSCalibur flow cytometer using FACSCalibur software (Becton Dickinson, Franklin Lakes, NJ). Only cultures that were >95% pure were used in our experiments.

DNA isolation and real-time PCR. DNA was isolated using Trizol, as recommended by the manufacturer (Invitrogen, Carlsbad, CA) from IFN-γ-stimulated or untreated U87MG cells and primary HFA and quantitated by conventional light absorption at 260/280 nm using a spectrophotometer. For each real-time PCR, 100 ng of DNA was amplified using a PCR mix containing 0.05 μM each of forward and reverse primers, 1× SYBR green, 1.5 mM $MgCl_2$, 0.25 mM deoxynucleoside triphosphates, and 0.02 U/μl *Taq* polymerase (Applied Biosystems, Foster City, CA). The primers used were R/U5 to amplify early reverse transcripts, R/5NC to amplify late reverse transcripts (24), SK145/150 to amplify *gag*/*pol* DNA (4b), or TAR1/TAR2 to amplify the bp 463 to 615 TAR HIV genomic sequence (accession no. K03455). Primer sequences for TAR1/TAR2 are TGGTTAGACCAGATCTGAGCC and TGACTAAAAGGGTCTGAGGGA, respectively. For quantitative SYBR green real-time PCR, the J1.1 cell line (NIH AIDS Research and Reference Reagents Program, Germantown, MD), which contains one proviral copy of HIV DNA per cell, was used as the HIV DNA standard at 2.5 × 10^1, 2.5 × 10^2, 2.5 × 10^3, 2.5 × 10^4, 2.5 × 10^5, and 2.5 × 10^6 copies per reaction. The amplification reaction consisted of an initial step at 94°C for 10 min and then 40 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 10 min.

ChIP assay. The formaldehyde cross-linking and chromatin immunoprecipitation (ChIP) assays of astrocytes were performed as described previously (14). Briefly, 5 × 10^6 cells per immunoprecipitation (IP) were used. TCF-4 antibody was purchased from Upstate (Charlottesville, VA). Conditions for the ChIP included amplification of the desired target, as indicated, after formaldehyde fixation of the samples but prior to any IP step, IP without the addition of an antibody, or IP using TCF-4 antibody. Pulled-down products underwent real-time PCR amplification using the SYBR green PCR kit (Applied Biosystems, Foster City, CA) and primer sequences and PCR conditions as indicated above.

Transfection. Primary human astrocytes and U87MG cells were transiently transfected using nucleofection, as recommended by the manufacturer (Amaxa, Gaithersburg, MD). Briefly, 5 × 10^6 cells were transfected with 10 μg of TOPflash, consisting of native TCF/LEF binding sites linked to a luciferase reporter vector (Upstate, Billerica, MA), 1 ng *Renilla* construct (Promega, Madison, WI), or green fluorescent protein (GFP) (pMaxGFP) construct as a control for transfection efficiency and to equalize the total amount of DNA used per transfection condition. Transfected cells were then left untreated or treated with IFN-γ, and luciferase reporter activity was evaluated 24 h later by the dual luciferase assay, as recommended by the manufacturer (Promega, Madison, WI). Luciferase values were normalized to *Renilla* activity. In some experiments, 3 × 10^6 to 5 × 10^6 cells were transfected with a TCF-4 dominant-negative mutant (James O'Kelly, UCLA, CA), which is a specific inhibitor of the β-catenin/TCF-4 complex (49), or with pMaxGFP, prior to infection with HIV, and p24 levels were measured at day 7 postinfection.

Statistical analysis. Descriptive statistics and graphical analysis were used. Nonparametric tests, such as the Wilcoxon rank sum test, were used as appropriate. GraphPad Instat software was used for data analysis.

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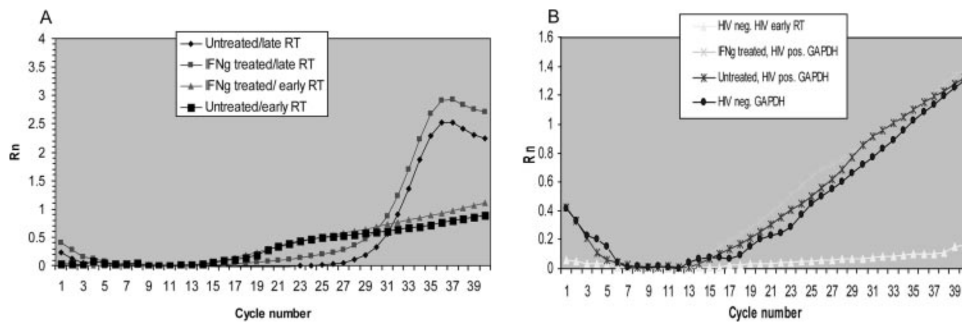

FIG. 1. Comparative analysis of early and late HIV reverse transcription (RT) between infected untreated and infected IFN-γ-treated astrocytes. U87MG cells were left untreated or treated with IFN-γ prior to HIV infection as described in Materials and Methods. Total DNA was isolated and amplified for either early HIV reverse transcription (reverse transcription initiation) using primer pair R and U5 at 24 h postinfection or late HIV transcription using primer pair R and 5NC at 96 h postinfection. (A) Comparison of amplified HIV DNA between untreated and IFN-γ-treated cultures. (B) All of the controls for real-time PCR including GAPDH amplification from HIV-positive (pos.) and -negative (neg.) cultures. Data shown are representative of at least two experiments. In Fig. 1, 2, and 3, Rn stands for reading normalized, which equals the SyberGreen value divided by the ROX reference dye value.

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RESULTS

Levels of HIV reverse transcription are similar between untreated and IFN-γ-treated astrocytes. We previously established that IFN-γ pretreatment of astrocytes leads to induction of HIV replication (7). To evaluate the mechanism of HIV restriction in astrocytes and the mechanism by which IFN-γ regulates this restriction, we compared the rates of HIV early and late reverse transcription between untreated and IFN-γ-treated astrocytes. U87MG cells were left untreated or pretreated with IFN-γ for 24 h and then infected with HIV_{BAL}. Unbound virus was removed by trypsinization. Early HIV reverse transcription was evaluated by real-time PCR at 24 h postinfection by amplification with the R/U5 primer pair, which detects negative-strand "strong-stop" DNA indicative of reverse transcription initiation (16, 24). Late HIV reverse transcription was measured 96 h postinfection using primer pair R/5NC, which amplifies late reverse transcripts containing positive-strand DNA after the second template switch beyond the primer binding site (16, 24). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was coamplified as an internal control. The levels of early and late reverse transcription DNA amplification were similar between cultures left untreated and those treated with IFN-γ (Fig. 1). These data indicate that HIV enters astrocytes and undergoes early and late reverse transcription but that enhanced virion entry or an accelerated rate of reverse transcription post-IFN-γ treatment is not a likely contributing factor for IFN-γ-mediated induction of HIV replication.

The HIV LTR in untreated astrocytes is associated with acetylated histones indicative of regions of active gene transcription. Given that HIV undergoes early and late reverse transcription in astrocytes and yet is well documented to be restricted in productive HIV replication (22), we evaluated the status of histone modification of the HIV LTR in untreated astrocytes. Several histones wrap around the DNA, and when deacetylated they lead to chromatin condensation and are associated with inactive genes (29, 34, 52). However, once the histones are acetylated, the chromatin structure is modified, becoming accessible to replication enzymes. Acetylated histones correlate with regions of active gene expression. For instance, multiple acetylated lysine residues have been identified in the N-terminal domain of H2B that correlate with gene-specific transcriptional activation (38). These modifications aid in the structural and functional properties of nucleosome and nucleosomal arrays seen in various activated cellular genes (2). We therefore hypothesized that inhibition of HIV replication in astrocytes may be due to its association with histone modification (i.e., deacetylation), leading to inactive gene transcription. To assess this hypothesis, a ChIP assay was performed on U87MG cells infected with HIV and DNA was analyzed 96 h postinfection. An antibody to acetylated histone H2B (Lys 5/12/15/20) was used to immunoprecipitate cellular DNA from HIV-infected astrocytes. These complexes were then dissociated, and the DNA was amplified for the HIV LTR at the region between −460 and −206 and the R and U5 regions including TAR between +1 and +153 from the transcription initiation site. Detection of a signal indicates association with the acetylated protein. We demonstrate that the two regions amplified for LTR and the TAR-spanning region were associated with acetylated histones (Fig. 2), as their respective DNA was amplified post-IP with the acetylated-histone-recognizing antibody. These data indicate that the HIV promoters at two loci (+1 to +153 and −460 to −206) are associated with transcription-ready complexes. The fact that the immunoprecipitated DNA contained both HIV-specific genes and the housekeeping gene (GAPDH), suggests that the HIV DNA amplified in these cultures represented integrated DNA. These data further suggest that the block to restricted HIV replication in astrocytes is not caused by condensed chromatin association and subsequent inhibition of active HIV gene transcription.

An inverse association between TCF-4 IP with HIV TAR-containing region and productive HIV replication in astrocytes. TCF-4, a Wnt signaling transcriptional factor, is defined as a transcriptional repressor of the HIV LTR. Although the exact mechanism of TCF-4-mediated inhibition of the HIV LTR is not clearly delineated, its association with HIV Tat may inhibit Tat transactivation of the HIV LTR (55). To evaluate the effect of TCF-4 on both HIV restriction in astrocytes and IFN-γ-mediated induction of HIV replication in astrocytes, the

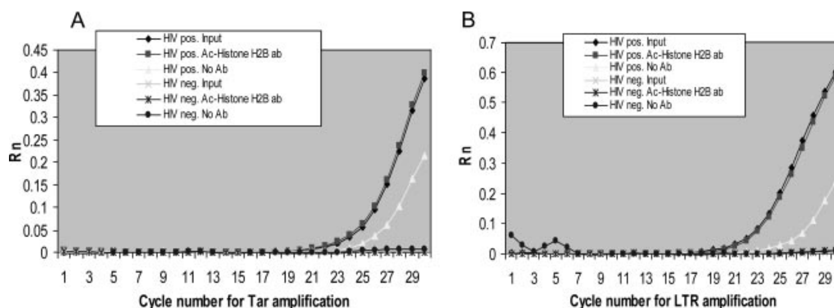

FIG. 2. Chromatin structures of TAR and LTR sequences are competent for transcriptional activities. U87MG cells were HIV infected or left uninfected. At 96 h, ChIP was performed by immunoprecipitating DNA associated with acetylated histones by using acetylated histone H2B antibody or no antibody. The immunoprecipitated DNA was subsequently amplified for TAR sequences between genomic locations +1 and +153 (A) and LTR sequences between genomic locations −460 and −206 (B). Conventional ChIP assay controls were included and shown in this figure, such as an "input control," referring to amplification of the DNA before the IP step, and a "no antibody control," referring to amplification of DNA after the IP step but without the addition of an acetylated histone H2B antibody. Data are representative of at least three experiments. Ac, acetylated; Ab and ab, antibody; pos., positive; neg., negative.

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association between TCF-4 and the HIV TAR-containing region was examined with or without IFN-γ treatment. ChIP was performed on U87MG cells left untreated or treated with IFN-γ, infected with HIV, chromatin immunoprecipitated with a TCF-4-specific antibody, and DNA amplified for HIV between +1 and +153 bp. We demonstrate that TCF-4 is immunoprecipitated with TAR in untreated cultures but that this association is absent when the cells are primed with IFN-γ (Fig. 3). Taken with our observation and that of others that untreated astrocytes do not support productive HIV replication, whereas IFN-γ priming induces HIV replication (7), these data suggest that there is an inverse relationship between TCF-4 association with TAR-containing region and HIV replication. It is still unclear if TCF-4 is physically bound to DNA elements in R and U5 regions of HIV TAR or to another protein that subsequently is bound to TAR. In the latter case, TCF-4 IP will also precipitate the entire multiprotein complex. These and previously published data (55) point to a role for TCF-4 in regulating HIV replication in astrocytes.

TCF-4 inhibition reverses the restriction of HIV replication in untreated/non-cytokine-primed U87MG cells and primary HFA. Given the observed inverse relationship between HIV replication in astrocytes and TCF-4 and TAR association, whether via direct or indirect binding, we evaluated the direct role of TCF-4 in HIV replication by using a dominant-negative mutant of TCF-4. This dominant-negative TCF-4 mutant is mutated in its β-catenin binding site and is a repressor of TCF-4 activity through the canonical/β-catenin-dependent pathway (39, 49). U87MG cells and primary HFA were transfected with a TCF-4 dominant-negative mutant or GFP-encoding plasmid. The total amount of DNA remained constant between the cultures. Twenty-four hours posttransfection, U87MG cells and HFA were infected with HIV, and HIV replication was measured by p24 ELISA on day 7. The efficiency of transfection at day 3 posttransfection was approximately 50% and 35% for U87MG cells and HFA, respectively, as measured by GFP expression (Fig. 4A). Inhibiting TCF-4 in U87MG cells and HFA modulated HIV replication by three-

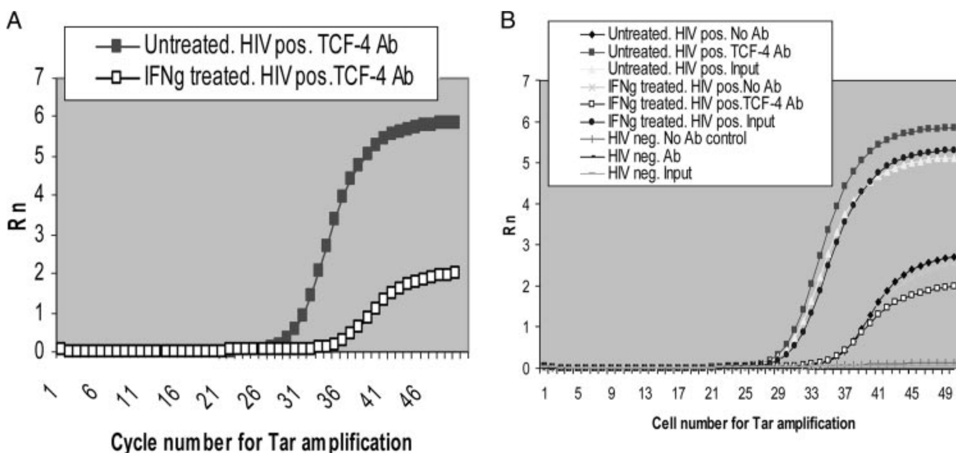

FIG. 3. TCF-4 is immunoprecipitated with HIV TAR-containing region in untreated but not IFN-γ-treated cultures. U87MG cells were left untreated or primed with IFN-γ, as described previously (7), and infected with HIV, and ChIP was performed using TCF-4 antibody for IP. (A) Comparison of untreated and IFN-γ-treated TAR-containing HIV DNA immunoprecipitated with TCF-4. (B) Additional controls of uninfected cultures, input DNA, and no-antibody controls. pos., positive; neg., negative; Ab, antibody.

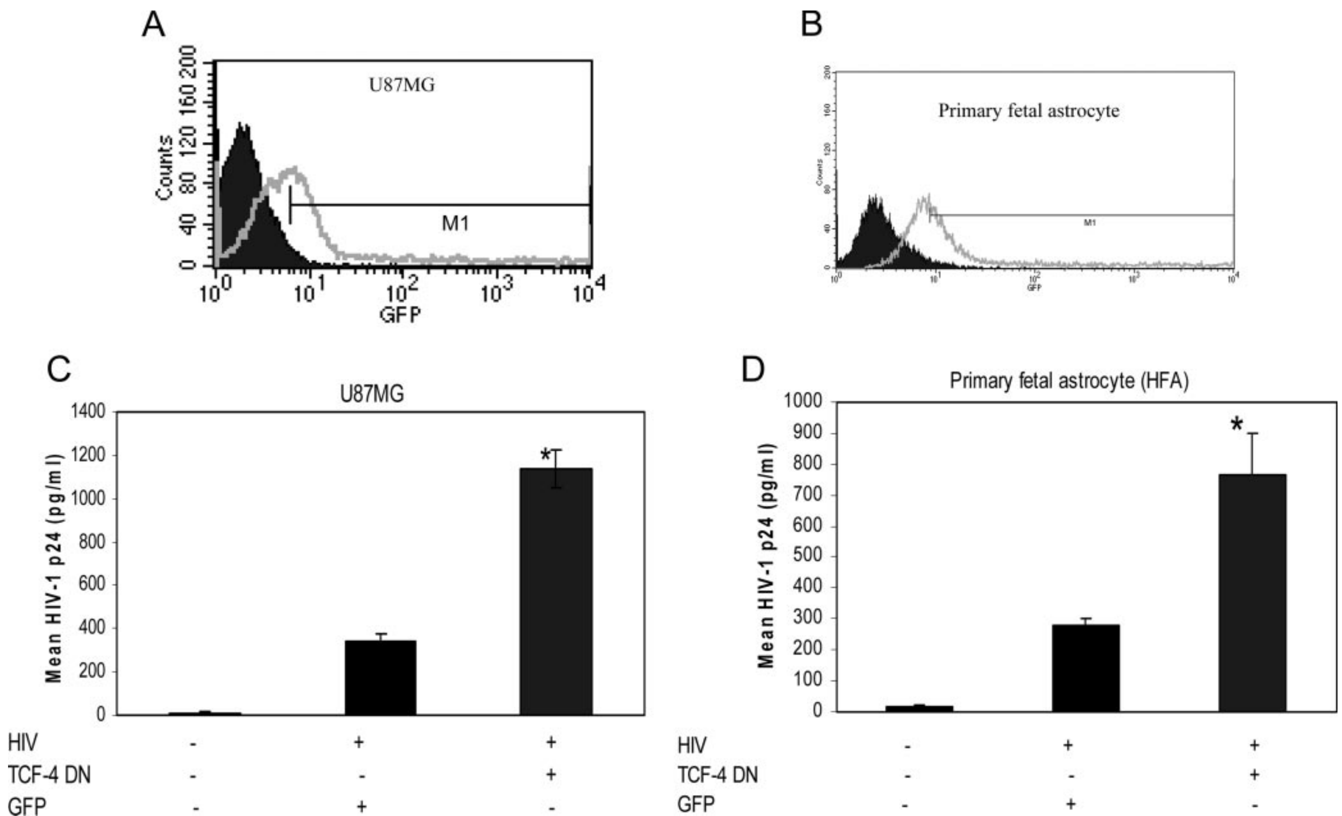

FIG. 4. (A and B) Transfection efficiencies of U87MG cells (A) and HFA (B). The cells were transfected with GFP (pMaxGFP) plasmid using the Nucleofector system (Amaxa). Expression of GFP was evaluated by flow cytometry at day 3 posttransfection. The black histogram represents an isotype control, and the gray histogram represents GFP expression. TCF-4 inhibition abrogates restriction of HIV replication in untreated/non-IFN-γ-primed cells. (C and D) U87MG cells (C) or primary HFA (D) were transfected with the TCF-4 dominant-negative (DN) mutant or GFP construct, keeping the DNA amount constant between the two cultures. The cells were then infected with HIV. Data represent mean HIV p24 levels (pg/ml) ± standard errors of the means. HIV p24 level was measured by conventional ELISA at day 7 postinfection. Asterisks in panels C and D indicate $P = 0.008$ and $P = 0.002$, respectively, calculated using the Wilcoxon rank sum test between dominant-negative TCF-4- and GFP-transfected cells. Cultures infected with HIV without GFP transfection had p24 values similar to those of HIV-infected, GFP-transfected cultures, as we previously reported (7).

fold in comparison to cells transfected with the GFP-encoding plasmid alone (Fig. 4B). This level of HIV replication after transfection of astrocytes with dominant-negative TCF-4 is similar to that achieved by priming the cells with IFN-γ, as we previously reported (7). Priming the cells with IFN-γ prior to transfection with dominant-negative TCF-4 did not result in a higher rate of HIV replication than that in cultures treated with IFN-γ alone (data not shown). These data indicate that inhibition of TCF-4 activity removes the restriction on HIV replication in astrocytes.

Active Wnt signaling in astrocytes and its inhibition by IFN-γ. Although inhibition of TCF-4 by the dominant-negative mutant stresses the importance of active TCF-4 in restricting HIV replication, it was not informative regarding the mechanism by which IFN-γ overcomes astrocyte restriction to productive HIV replication. To evaluate the impact of IFN-γ on TCF-4 activity in regulating HIV replication, U87MG cells were left untreated or IFN-γ treated and then transfected with either a TCF-4 luciferase construct (TOPflash) or a GFP construct and cultured with or without IFN-γ. The TCF-4 reporter construct is an indicator of basal and inducible levels of Wnt signaling (45). Basal TCF-4 activity was detected in astrocytes, indicating active Wnt signaling in human astrocytes (Fig. 5). IFN-γ markedly reduced this signal by approximately 50% (Fig. 5). These data in conjunction with the TCF-4 ChIP and dominant-negative transfection data indicate that active Wnt signaling is associated with HIV restriction in astrocytes and that IFN-γ overcomes this restriction by reducing the potency of this pathway. This is the first indication that IFN-γ regulates Wnt signaling, which can be harnessed to restrict HIV replication.

DISCUSSION

Cell types that limit HIV replication are beneficial in understanding molecular mechanisms of innate/intrinsic restriction to HIV replication. Astrocytes limit HIV replication very successfully (23), and this can serve as a model to restrict HIV replication within and outside of the brain. At least four mechanisms have been proposed for the postentry block of productive HIV infection in astrocytes. (i) One is defects in Rev function due to low constitutive expression of an essential protein for Rev function known as Sam68 (26, 27, 57). (ii) Another is a block downstream of Rev-mediated transport at

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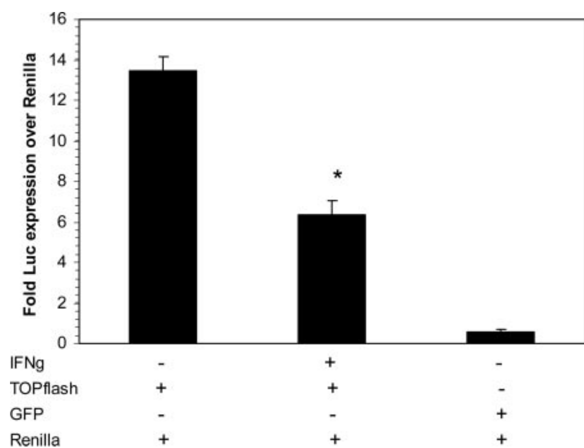

FIG. 5. Impact of IFN-γ on Wnt signaling. U87MG cells were left untreated or treated with IFN-γ for 24 h and then transfected with either TOPflash (containing four native TCF/LEF binding sites) and *Renilla* constructs or GFP and *Renilla* constructs. Transfected cells were then cultured with or without their initial treatment (with or without IFN-γ). Luciferase relative light activity (relative light units) was measured 24 h later using a luminometer and normalized to *Renilla* activity. Data are based on at least three experiments and are presented as the increase (*n*-fold) in luciferase relative light units over *Renilla* ± standard deviation. The asterisk shows $P < 0.0001$ between untreated (first column) and IFN-γ-treated (second column) cultures using the Bonferroni multiple comparison test.

the level of translation initiation whereby enhanced double-stranded RNA-activated protein kinase expression in astrocytes is associated with a block in protein synthesis of TAR RNA binding protein. TAR RNA binding protein in permissive targets increases HIV expression by blocking RNA-activated protein kinase activity, enhancing viral translation, and mediating the translation of TAR-encoding RNAs (4, 13, 15, 37). (iii) Another is trafficking of HIV to endosomes by binding to the mannose receptor, which targets HIV for degradation (30). Low-level HIV replication in this model is explained by the occasional escape of HIV from this endocytic trafficking compartment (30). (iv) Last is repression of HIV transcription by TCF-4 (55). Even though we demonstrate a physiologic role for TCF-4 in repressing HIV replication in astrocytes, these studies are not in contrast to the other models proposed for HIV restriction. Despite inhibition of TCF-4 activity, astrocytes still express less HIV than activated T cells do. Other factors contribute to the repression of HIV in these cells, supporting the hypothesis that there may be a bottleneck for virus production in these cells resulting from multiple blocks during the viral life cycle (12). The relative importance of the blocks at each of these steps requires further analyses. Additionally, because TCF-4 is the downstream effector of the Wnt signaling pathway, which regulates the expression of a number of genes, the Wnt pathway may converge on the various models proposed for restricted HIV replication in astrocytes.

The exact mechanism of action of the TCF-4 transcription factor in inhibiting HIV infection is still not clear. Wortman et al. (55) reported that TCF-4 is a repressor of basal and Tat-mediated transactivation of the HIV LTR. This repression was reported to occur in a β-catenin-independent manner. Thus, the core data are not different in showing that TCF-4 is a repressor of HIV replication, but the interpretation of how this may occur varies. We believe that this is β-catenin dependent, and Wortman et al. indicate that it is not. Our model is more physiologic because it is based on basal levels of TCF-4 within astrocytes and infection of whole virus rather than transfecting the cells with exogenous TCF-4 and Tat as was done previously (55). Wortman et al. further suggested that TCF-4 binds directly to Tat, possibly sequestering it from binding to TAR (55). However, we show here that TCF-4 is complexed with a region that spans TAR (+1 to +153). It is also still likely that TCF-4 may bind Tat and that this complex binds to or near TAR, altering the stereochemistry of Tat-TAR interaction, leading to HIV inhibition or the formation of a multicomplex among TCF-4/β-catenin, Tat, and TAR that leads to HIV inhibition. Recently it was also shown that TCF-4 interacts with the Sp1 transcription factor, which is one of the transcription factors that activate the HIV LTR, interfering with Sp1 LTR transactivation ability, in the absence of Tat (40). This interference could therefore result in lowered HIV gene transcription. These recent data point to a multi-DNA binding protein complex that inhibits HIV replication, the exact components of which remain to be elucidated. Our finding that astrocytes may be responding in an autocrine manner to Wnt activating ligands is not surprising, since the secretion of these Wnt proteins by astrocytes is known to occur as part of their role in maintaining homeostasis of the neuronal population (28). Collectively, our findings demonstrate that Wnt activation in astrocytes underlies a significant block to HIV replication.

The ability of the dominant-negative mutant of TCF-4 to elude the restriction of HIV replication in astrocytes and the fact that the dominant-negative TCF-4 is mutated in its β-catenin binding site suggest that the association between β-catenin and TCF-4 is critical for suppressing HIV replication and that repression of HIV replication probably occurs via the canonical/β-catenin-dependent Wnt signaling pathway. The mechanism of IFN-γ-mediated downregulation of this HIV-inhibitory complex may also be multifaceted. While IFN-γ is known to increase phosphorylation and binding of Sp1 (43), which could in turn bind TCF-4, sequestering it from binding the HIV LTR, we show a link between IFN-γ and the downstream effector of Wnt activity (TCF/LEF transcription). Although IFN-γ inhibited TCF/LEF transcriptional activity, this inhibition may be indirect through inhibition of β-catenin, which is important in regulating the transcription of TCF genes. Alternatively, classical signaling of IFN-γ through the Stat-Jun pathway may be related to Wnt signaling, leading to the observed effect of IFN-γ markedly reducing TCF/LEF transcriptional activity. A role for IFN-γ in inhibiting the Wnt pathway is further supported by studies showing that IFN-γ upregulates the secretion of Wnt inhibitor DKK1, albeit in human melanoma cell lines (21).

These studies indicate that active Wnt signaling in astrocytes is associated with restricted HIV replication. In the setting of chronic inflammation that occurs during HIV infection of the central nervous system, IFN-γ expression is elevated and is associated with HIV-associated dementia/encephalitis (25). Expression of IFN-γ can thus compromise the innate ability of astrocytes to resist productive HIV replication, through the observed inhibition of Wnt signaling. Numerous examples exist

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of HIV manipulating the immune system in its favor, as in its ability to downregulate CD4 and antigen presentation molecules (major histocompatibility complex classes I and II and CD1d) (8, 11). This theme may emerge again whereby IFN-γ upregulates major histocompatibility complex class II on astrocytes (data not shown and data reported in references 6 and 20), possibly enhancing their antigen presentation capability and immune competence, but at the same time IFN-γ may inactivate the intrinsic pathway (Wnt signaling) that restricts HIV replication in astrocytes, leading to their susceptibility to HIV productive infection. Wnt signaling through β-catenin is a survival signal as it is associated with upregulation of the antiapoptotic gene product Bcl-xL (56). IFN-γ, conversely, is a proapoptotic signal for virally infected cells. Therefore, IFN-γ-mediated inhibition of the Wnt pathway seems a logical consequence if the net effect of IFN-γ is cell death rather than survival of the infected target. HIV may take advantage of this pathway by replicating in cells receiving an IFN-γ signal that inhibits Wnt signaling. These studies are thus critical to gaining an understanding of factors within the brain microenvironment that may regulate HIV replication within astrocytes and consequently susceptibility to HIV-associated cognitive-motor impairment. Dysregulated astrocytes lead to significant biologic abnormalities in the brain, such as dysregulated neurotoxin scavenging capabilities and/or maintenance of the blood-brain barrier by astrocytes, leading to clinical manifestation of HIV-associated neuropathogenesis (23, 32, 41, 53).

Based on our data demonstrating the ability of TCF-4 to restrict HIV replication and those published by others pointing to TCF-4 as an HIV repressor (55), we suggest that the Wnt pathway, which regulates TCF-4 activity, is an intracellular innate pathway that restricts HIV replication. To our knowledge this is the first rationale for a signaling pathway that can be easily activated and/or inactivated with commercially available reagents to influence HIV replication within and outside of the brain. Ultimately, arsenals of natural host restrictive proteins (APOPEC 3G, TRIM5a, and Wnt pathway) can be translated as new modalities for anti-HIV therapy.

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REFERENCES

1. **An, S. F., M. Groves, B. Giometto, A. A. Beckett, and F. Scaravilli.** 1999. Detection and localisation of HIV-1 DNA and RNA in fixed adult AIDS brain by polymerase chain reaction/in situ hybridisation technique. Acta Neuropathol. (Berlin) **98:**481–487.
2. **Angelov, D., A. Verdel, W. An, V. Bondarenko, F. Hans, C. M. Doyen, V. M. Studitsky, A. Hamiche, R. G. Roeder, P. Bouvet, and S. Dimitrov.** 2004. SWI/SNF remodeling and p300-dependent transcription of histone variant H2ABbd nucleosomal arrays. EMBO J. **23:**3815–3824.
3. **Atwood, W. J., C. S. Tornatore, R. Traub, K. Conant, P. D. Drew, and E. O. Major.** 1994. Stimulation of HIV type 1 gene expression and induction of NF-kappa B (p50/p65)-binding activity in tumor necrosis factor alpha-treated human fetal glial cells. AIDS Res. Hum. Retrovir. **10:**1207–1211.
4. **Bannwarth, S., and A. Gatignol.** 2005. HIV-1 TAR RNA: the target of molecular interactions between the virus and its host. Curr. HIV Res. **3:**61–71.

4a. **Bencheikh, M., G. Bentsman, N. Sarkissian, M. Canki, and D. J. Volsky.** 1999. Replication of different clones of human immunodeficiency virus type 1 in primary fetal human astrocytes: enhancement of viral gene expression by Nef. J. Neurovirol. **5:**115–124.

4b. **Brandt, C. D., T. A. Rakusan, A. V. Sison, S. H. Josephs, E. S. Saxena, K. D. Herzog, R. H. Parrott, and J. L. Sever.** 1992. Detection of human immunodeficiency virus type 1 infection in young pediatric patients by using polymerase chain reaction and biotinylated probes. J. Clin. Microbiol. **30:**36–40.

5. **Canki, M., J. N. Thai, W. Chao, A. Ghorpade, M. J. Potash, and D. J. Volsky.** 2001. Highly productive infection with pseudotyped human immunodeficiency virus type 1 (HIV-1) indicates no intracellular restrictions to HIV-1 replication in primary human astrocytes. J. Virol. **75:**7925–7933.
6. **Carpentier, P. A., W. S. Begolka, J. K. Olson, A. Elhofy, W. J. Karpus, and S. D. Miller.** 2005. Differential activation of astrocytes by innate and adaptive immune stimuli. Glia **49:**360–374.
7. **Carroll-Anzinger, D., and L. Al-Harthi.** 2006. Gamma interferon primes productive human immunodeficiency virus infection in astrocytes. J. Virol. **80:**541–544.
8. **Chen, N., C. McCarthy, H. Drakesmith, D. Li, V. Cerundolo, A. J. McMichael, G. R. Screaton, and X. N. Xu.** 2006. HIV-1 down-regulates the expression of CD1d via Nef. Eur. J. Immunol. **36:**278–286.
9. **Chen, Y., N. E. Vartiainen, W. Ying, P. H. Chan, J. Koistinaho, and R. A. Swanson.** 2001. Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. J. Neurochem. **77:**1601–1610.
10. **Cheng-Mayer, C., J. T. Rutka, M. L. Rosenblum, T. McHugh, D. P. Stites, and J. A. Levy.** 1987. Human immunodeficiency virus can productively infect cultured human glial cells. Proc. Natl. Acad. Sci. USA **84:**3526–3530.
11. **Cho, S., K. S. Knox, L. M. Kohli, J. J. He, M. A. Exley, S. B. Wilson, and R. R. Brutkiewicz.** 2005. Impaired cell surface expression of human CD1d by the formation of an HIV-1 Nef/CD1d complex. Virology **337:**242–252.
12. **Clarke, J. N., J. A. Lake, C. J. Burrell, S. L. Wesselingh, P. R. Gorry, and P. Li.** 2006. Novel pathway of human immunodeficiency virus type 1 uptake and release in astrocytes. Virology **348:**141–155.
13. **Daher, A., M. Longuet, D. Dorin, F. Bois, E. Segeral, S. Bannwarth, P. L. Battisti, D. F. Purcell, R. Benarous, C. Vaquero, E. F. Meurs, and A. Gatignol.** 2001. Two dimerization domains in the trans-activation response RNA-binding protein (TRBP) individually reverse the protein kinase R inhibition of HIV-1 long terminal repeat expression. J. Biol. Chem. **276:**33899–33905.
14. **de La Fuente, C., L. Deng, F. Santiago, L. Arce, L. Wang, and F. Kashanchi.** 2000. Gene expression array of HTLV type 1-infected T cells: up-regulation of transcription factors and cell cycle genes. AIDS Res. Hum. Retrovir. **16:**1695–1700.
15. **Dorin, D., M. C. Bonnet, S. Bannwarth, A. Gatignol, E. F. Meurs, and C. Vaquero.** 2003. The TAR RNA-binding protein, TRBP, stimulates the expression of TAR-containing RNAs in vitro and in vivo independently of its ability to inhibit the dsRNA-dependent kinase PKR. J. Biol. Chem. **278:**4440–4448.
16. **Dornadula, G., S. Yang, R. J. Pomerantz, and H. Zhang.** 2000. Partial rescue of the Vif-negative phenotype of mutant human immunodeficiency virus type 1 strains from nonpermissive cells by intravirion reverse transcription. J. Virol. **74:**2594–2602.
17. **Dorsky, R. I., R. T. Moon, and D. W. Raible.** 2000. Environmental signals and cell fate specification in premigratory neural crest. Bioessays **22:**708–716.
18. **Epstein, L. G., L. R. Sharer, E. S. Cho, M. Myenhofer, B. Navia, and R. W. Price.** 1984. HTLV-III/LAV-like retrovirus particles in the brains of patients with AIDS encephalopathy. AIDS Res. **1:**447–454.
19. **Fiala, M., R. H. Rhodes, P. Shapshak, I. Nagano, O. Martinez-Maza, A. Diagne, G. Baldwin, and M. Graves.** 1996. Regulation of HIV-1 infection in astrocytes: expression of Nef, TNF-alpha and IL-6 is enhanced in coculture of astrocytes with macrophages. J. Neurovirol. **2:**158–166.
20. **Girvin, A. M., K. B. Gordon, C. J. Welsh, N. A. Clipstone, and S. D. Miller.** 2002. Differential abilities of central nervous system resident endothelial cells and astrocytes to serve as inducible antigen-presenting cells. Blood **99:**3692–3701.
21. **Gollob, J. A., C. J. Sciambi, Z. Huang, and H. K. Dressman.** 2005. Gene expression changes and signaling events associated with the direct antimelanoma effect of IFN-gamma. Cancer Res. **65:**8869–8877.
22. **Gorry, P. R., J. L. Howard, M. J. Churchill, J. L. Anderson, A. Cunningham, D. Adrian, D. A. McPhee, and D. F. Purcell.** 1999. Diminished production of human immunodeficiency virus type 1 in astrocytes results from inefficient translation of *gag*, *env*, and *nef* mRNAs despite efficient expression of Tat and Rev. J. Virol. **73:**352–361.
23. **Gorry, P. R., C. Ong, J. Thorpe, S. Bannwarth, K. A. Thompson, A. Gatignol, S. L. Vesselingh, and D. F. Purcell.** 2003. Astrocyte infection by HIV-1: mechanisms of restricted virus replication, and role in the pathogenesis of HIV-1-associated dementia. Curr. HIV Res. **1:**463–473.
24. **Kameoka, M., L. Rong, M. Gotte, C. Liang, R. S. Russell, and M. A. Wainberg.** 2001. Role for human immunodeficiency virus type 1 Tat protein in suppression of viral reverse transcriptase activity during late stages of viral replication. J. Virol. **75:**2675–2683.
25. **Langford, D., and E. Masliah.** 2001. Crosstalk between components of the blood brain barrier and cells of the CNS in microglial activation in AIDS. Brain Pathol. **11:**306–312.
26. **Li, J., Y. Liu, B. O. Kim, and J. J. He.** 2002. Direct participation of Sam68, the 68-kilodalton Src-associated protein in mitosis, in the CRM1-mediated Rev nuclear export pathway. J. Virol. **76:**8374–8382.
27. **Li, J., Y. Liu, I. W. Park, and J. J. He.** 2002. Expression of exogenous Sam68, the 68-kilodalton SRC-associated protein in mitosis, is able to alleviate impaired Rev function in astrocytes. J. Virol. **76:**4526–4535.
28. **Lie, D. C., S. A. Colamarino, H. J. Song, L. Desire, H. Mira, A. Consiglio,**

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E. S. Lein, S. Jessberger, H. Lansford, A. R. Dearie, and F. H. Gage. 2005. Wnt signalling regulates adult hippocampal neurogenesis. Nature **437:**1370–1375.

29. **Liu, T., S. Kuljaca, A. Tee, and G. M. Marshall.** 2006. Histone deacetylase inhibitors: multifunctional anticancer agents. Cancer Treat. Rev. **32:**157–165.
30. **Liu, Y., H. Liu, B. O. Kim, V. H. Gattone, J. Li, A. Nath, J. Blum, and J. J. He.** 2004. CD4-independent infection of astrocytes by human immunodeficiency virus type 1: requirement for the human mannose receptor. J. Virol. **78:**4120–4133.
31. **Ludwig, E., F. Ceccherini-Silberstein, J. van Empel, V. Erfle, M. Neumann, and R. Brack-Werner.** 1999. Diminished Rev-mediated stimulation of human immunodeficiency virus type 1 protein synthesis is a hallmark of human astrocytes. J. Virol. **73:**8279–8289.
32. **Managlia, E. Z., D. Carroll, A. Zloza, and L. Al-Harthi.** 2004. Immune modulation of HIV replication: relevance to HIV immuno- and neuropathogenesis. Curr. HIV Res. **2:**395–401.
33. **Meyenhofer, M. F., L. G. Epstein, E. S. Cho, and L. R. Sharer.** 1987. Ultrastructural morphology and intracellular production of human immunodeficiency virus (HIV) in brain. J. Neuropathol. Exp. Neurol. **46:**474–484.
34. **Moradei, O., C. R. Maroun, I. Paquin, and A. Vaisburg.** 2005. Histone deacetylase inhibitors: latest developments, trends and prospects. Curr. Med. Chem. Anticancer Agents **5:**529–560.
35. **Nath, A.** 2002. Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia. J. Infect. Dis. **186:**S193–S198.
36. **Neumann, M., B. K. Felber, A. Kleinschmidt, B. Froese, V. Erfle, G. N. Pavlakis, and R. Brack-Werner.** 1995. Restriction of human immunodeficiency virus type 1 production in a human astrocytoma cell line is associated with a cellular block in Rev function. J. Virol. **69:**2159–2167.
37. **Ong, C. L., J. C. Thorpe, P. R. Gorry, S. Bannwarth, A. Jaworowski, J. L. Howard, S. Chung, S. Campbell, H. S. Christensen, G. Clerzius, A. J. Mouland, A. Gatignol, and D. F. Purcell.** 2005. Low TRBP levels support an innate human immunodeficiency virus type 1 resistance in astrocytes by enhancing the PKR antiviral response. J. Virol. **79:**12763–12772.
38. **Parra, M. A., D. Kerr, D. Fahy, D. J. Pouchnik, and J. J. Wyrick.** 2006. Deciphering the roles of the histone H2B N-terminal domain in genome-wide transcription. Mol. Cell. Biol. **26:**3842–3852.
39. **Roose, J., and H. Clevers.** 1999. TCF transcription factors: molecular switches in carcinogenesis. Biochim. Biophys. Acta **1424:**M23–M37.
40. **Rossi, A., R. Mukerjee, P. Ferrante, K. Khalili, S. Amini, and B. E. Sawaya.** 2006. Human immunodeficiency virus type 1 Tat prevents dephosphorylation of Sp1 by TCF-4 in astrocytes. J. Gen. Virol. **87:**1613–1623.
41. **Sabri, F., K. Titanji, A. De Milito, and F. Chiodi.** 2003. Astrocyte activation and apoptosis: their roles in the neuropathology of HIV infection. Brain Pathol. **13:**84–94.
42. **Sabri, F., E. Tresoldi, M. Di Stefano, S. Polo, M. C. Monaco, A. Verani, J. R. Fiore, P. Lusso, E. Major, F. Chiodi, and G. Scarlatti.** 1999. Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors. Virology **264:**370–384.
43. **Sanceau, J., T. Kaisho, T. Hirano, and J. Wietzerbin.** 1995. Triggering of the human interleukin-6 gene by interferon-gamma and tumor necrosis factor-alpha in monocytic cells involves cooperation between interferon regulatory factor-1, NF kappa B, and Sp1 transcription factors. J. Biol. Chem. **270:**27920–27931.

43a. **Schweighardt, B., and W. J. Atwood.** 2001. HIV type 1 infection of human astrocytes is restricted by inefficient viral entry. AIDS Res. Hum. Retrovir. **7:**1133–1142.

44. **Shahabuddin, M., G. Bentsman, B. Volsky, I. Rodriguez, and D. J. Volsky.** 1996. A mechanism of restricted human immunodeficiency virus type 1 expression in human glial cells. J. Virol. **70:**7992–8002.
45. **Shulewitz, M., I. Soloviev, T. Wu, H. Koeppen, P. Polakis, and C. Sakanaka.** 2006. Repressor roles for TCF-4 and Sfrp1 in Wnt signaling in breast cancer. Oncogene **25:**4361–4369.
46. **Staal, F. J., J. Meeldijk, P. Moerer, P. Jay, B. C. van de Weerdt, S. Vainio, G. P. Nolan, and H. Clevers.** 2001. Wnt signaling is required for thymocyte development and activates Tcf-1 mediated transcription. Eur. J. Immunol. **31:**285–293.
47. **Tanaka, J., K. Toku, B. Zhang, K. Ishihara, M. Sakanaka, and N. Maeda.** 1999. Astrocytes prevent neuronal death induced by reactive oxygen and nitrogen species. Glia **28:**85–96.
48. **Timm, A., and R. Grosschedl.** 2005. Wnt signaling in lymphopoiesis. Curr. Top. Microbiol. Immunol. **290:**225–252.
49. **Tong, X., J. O'Kelly, D. Xie, A. Mori, N. Lemp, R. McKenna, C. W. Miller, and H. P. Koeffler.** 2004. Cyr61 suppresses the growth of non-small-cell lung cancer cells via the beta-catenin-c-myc-p53 pathway. Oncogene **23:**4847–4855.
50. **Tornatore, C., K. Meyers, W. Atwood, K. Conant, and E. Major.** 1994. Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. J. Virol. **68:**93–102.

50a. **Tornatore, C., A. Nath, K. Amemiya, and E. O. Major.** 1991. Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. J. Virol. **65:**6094–6100.

51. **Trillo-Pazos, G., A. Diamanturos, L. Rislove, T. Menza, W. Chao, P. Belem, S. Sadiq, S. Morgello, L. Sharer, and D. J. Volsky.** 2003. Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by laser capture microdissection. Brain Pathol. **13:**144–154.
52. **Varier, R. A., and T. K. Kundu.** 2006. Chromatin modifications (acetylation/deacetylation/methylation) as new targets for HIV therapy. Curr. Pharm. Des. **12:**1975–1993.
53. **Wang, Z., G. Trillo-Pazos, S. Y. Kim, M. Canki, S. Morgello, L. R. Sharer, H. A. Gelbard, Z. Z. Su, D. C. Kang, A. I. Brooks, P. B. Fisher, and D. J. Volsky.** 2004. Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: potential role in neuropathogenesis. J. Neurovirol. **10**(Suppl. 1):25–32.
54. **Wiley, C. A., R. D. Schrier, J. A. Nelson, P. W. Lampert, and M. B. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83:**7089–7093.
55. **Wortman, B., N. Darbinian, B. E. Sawaya, K. Khalili, and S. Amini.** 2002. Evidence for regulation of long terminal repeat transcription by Wnt transcription factor TCF-4 in human astrocytic cells. J. Virol. **76:**11159–11165.
56. **Xie, H., Z. Huang, M. S. Sadim, and Z. Sun.** 2005. Stabilized beta-catenin extends thymocyte survival by up-regulating Bcl-xL. J. Immunol. **175:**7981–7988.
57. **Zhang, J., Y. Liu, J. Henao, M. T. Rugeles, J. Li, T. Chen, and J. J. He.** 2005. Requirement of an additional Sam68 domain for inhibition of human immunodeficiency virus type 1 replication by Sam68 dominant negative mutants lacking the nuclear localization signal. Gene **363:**67–76.

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