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Increased In Vitro Glial Fibrillary Acidic Protein Expression, Telomerase Activity, and Telomere Length After Productive Human Immunodeficiency Virus-1 Infection in Murine Astrocytes

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Although HIV-associated neurocognitive disorders (HAND) result from injury and loss of neurons, productive infection routinely takes place in cells of macrophage lineage. In such a complex context, astrocytosis induced by local chemokines/cytokines is one of the hallmarks of HIV neuropathology. Whether this sustained astrocyte activation is able to alter telomere-aging process is unknown. We hypothesized that interaction of HIV with astrocytes may impact astrocyte telomerase activity (TA) and telomere length in a scenario of astrocytic activation measured by expression of glial fibrillary acidic protein (GFAP). To test this hypothesis, cultured murine astrocytes were challenged with pseudotyped HIV/vesicular stomatitis virus (HIV/VSV) to circumvent the absence of viral receptors; and GFAP, telomerase activity, and telomere length were quantified. As an early and transient event after HIV infection, both TA activity and telomere length were significantly augmented ($P < 0.001$). Later, a strong negative correlation (-0.8616, $P < 0.0001$) between virus production and telomerase activity was demonstrated. Once HIV production had reached a peak (7 dpi), the TA decreased, showing levels similar to those of noninfected cells. In contrast, the astrocyte became activated, exhibiting significantly increased levels of GFAP expression directly related to the level of HIV/VSV replication ($P < 0.0001$). Our results suggest that HIV-infected astrocytes exhibit early disturbance in their cellular functions, such as telomerase activity and telomere length, that may attenuate cell proliferation and enhance the astrocyte dysregulation, contributing to HIV neuropathogenesis. Understanding the mechanisms involved in HIV-mediated persistence by altering the telomere-related aging processes could aid in the development of therapeutic modalities for neurological complications of HIV infection.

Key words: HIV-1; astrocytes; GFAP; telomerase; telomere length

Human immunodeficiency virus type 1 (HIV-1) invades the central nervous system (CNS). Since the first years of the HIV/AIDS epidemic, neuropathological conditions observed in infected individuals during late stages of disease were attributed to direct effect of viral infection of brain, as later was confirmed by HIV isolation from brain tissues, detection of intrathecal antibody synthesis, and viral RNA and proviral DNA identification in brain tissues (Gras and Kaul, 2010; Eugenin et al., 2011; Williams et al., 2012). HIV-associated neurological syndromes were later defined as associated neurocognitive disorders (HAND), and three categories were recognized according to neuropsychological testing: HIV-associated dementia (HAD), mild neurocognitive disorder, and asymptomatic neurocognitive impairment (Antinori et al., 2007). After the introduction of highly active antiretroviral therapy (HAART), the incidence of HAD has declined dramatically (20–30% to 2–3%), but the two other categories can still be diagnosed in nearly half of patients (Clifford, 2008). As Kaul et al. (2005) reviewed, measurement of cognitive dysfunction in neuro-AIDS did not correlate well with the number of HIV-infected cells or viral antigens in the CNS; instead, they correspond

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better to increased numbers of microglia and astrocytes, evidence of excitotoxins, decreased synaptic and dendritic density, and selective loss of neurons. Since HAND symptoms are closely associated with damage to and loss of neurons, although such cells cannot be infected by HIV, other neuropathogenic mechanisms rather than the direct infection must be involved (Gonzalez-Scarano and Martin-Garcia, 2005; Kaul et al., 2005; Spudich and González Scarano, 2012). Mechanisms leading to neuronal death are still under discussion, but the main role of macrophages and microglia is well established (Gorry et al., 2003; Gonzalez-Scarano and Martin-Garcia, 2005). Although restrictive infection of astrocytes is not able to support ongoing HIV infection in brain, such glial cells would contribute to HAND through mechanisms of astrogliosis induced by local proinflammatory chemokines and cytokines. Such astrocytosis, characterized by increased expression of glial fibrillary acidic protein (GFAP), is a recognized hallmark of HIV infection of thee CNS (Zhou et al., 2004; Bell et al., 2006; Zou et al., 2007). Nevertheless, a direct link between astrocyte activation and neuron death is still under consideration.

Interestingly, astrocytes undergo a functional decline with age in vivo that parallels functional declines in vitro, showing a telomere-aging process (Pertusa et al., 2007; Bhat et al., 2012). HIV infection may affect the cellular life span by affecting the telomere length and telomerase activity. This enzyme is a reverse transcriptase that adds TTAGGG repeats to telomeres and thus maintains telomere length and prevents cellular senescence (Nandakumar and Cech, 2013). We and others have reported that HIV infection negatively affects telomerase activity (TA) in $CD4^+$ and $CD8^+$ T lymphocytes (Ballon et al., 2001; Franzese et al., 2007; Reynoso et al., 2010) but increases macrophages (Reynoso et al., 2012). During development of the CNS, telomerase activity is high in neural progenitor cells but then decreases as differentiation ensues (Fu et al., 2000). Given that telomerase activity and telomere length are tightly related to the resistance of cells to stress and injury, we analyzed their variation during HIV-mediated astrocyte activation.

MATERIALS AND METHODS

Isolation and Culture of Primary Murine Astrocytes

Astroglial cell cultures were obtained from the brains of newborn Balb/c mice as described previously (Berria and Lascano, 1985; Borda et al., 2004) and following animal protocols that were approved by the authors' institutional animal experimentation committee. Briefly, cortical hemispheres were harvested and neural tissues digested by 0.25% trypsin. Flasks (75 cm^2) were seeded with 1×10^6 cells/ml of growth medium (Dulbecco's modified Eagle medium [DMEM]-supplemented with 20% fetal bovine serum [FBS], glutamine, and antibiotiotics) twice for 1 hr to eliminate fibroblast contamination. By changing the supernatant within 24 hr, neurons were eliminated by means of differential attachment, leaving an almost homogenous population of glial cells. After 3 weeks at 37°C, the already confluent primary cell culture was shaken once for 2 hr at 37°C, and supernatant was discarded in order to detach contaminating oligodendrocytes growing on top of the remaining adherent astroglial cell monolayer, which in turn were trypsinized (Du et al., 2010). The resulting cells were then resuspended (5×10^6 cells/ml of growth medium) and seeded as first subculture in 24-well plates with 12-mm glass inserts for immunolabeling and in 25-cm^2 flasks for protein and DNA extraction. Subsequently, immunoperoxidase labeling for glial fibrillary acidic protein (GFAP) showed a highly homogeneous population of astrocytes (≥95%, data not shown). After 2 days of seeding, growth medium was removed, and cell monolayers were infected with HIV/VSV-G at a multiplicity of infection of 1. At 1, 6, 8, and 13 days postinfection (PI), three or four samples were harvested for each experimental time point. Supernatants were routinely frozen at −20°C for later measurement of HIV p24 antigen production. Monolayers were fixed with 2% paraformaldehyde plus 0.1% Triton X-100 at 37°C for immunolabeling or were trypsinized for protein and DNA extraction.

Generation of Pseudotyped HIV-1/VSV-G Virus and In Vitro Infection of Murine Astrocytes

Intact HIV-1 pseudotyped with the VSV-G glycoprotein was prepared by transfection of 293T cells as described previously (Canki et al., 2001). Briefly, the HIV-1 molecular clone used was NL4-3, which expresses all known HIV-1 proteins (Adachi et al., 1986). The VSV-G expression vector pL-VSV-G contains a VSV-G insert in the pcDNA expression vector modified by replacing the cytomegalovirus promoter with the HIV-1 long terminal repeat (Bartz et al., 1996). High-titer virus stocks were produced in 293T human embryonic kidney cells transfected with the appropriate DNA using Lipofectamine 2000 as recommended by the manufacturer (Ausubel et al., 1995). To generate pseudotyped virus, 1.5×10^6 293T cells cultured in 10-cm plates were cotransfected with 14 μg HIV-1 clone DNA and 7.5 μg VSV expression plasmid DNA with 3 μl Lipofectamine/μg DNA. 293T culture supernatants were harvested 72 hr after transfection, filtered through a 0.45-μm-pore Millipore filter, and stored at −80°C until use. Cell-free viral stock was tested for HIV-1 p24 core antigen content by ELISA using the HIV-1 Ag kit as specified by the manufacturer (Coulter, Hialeah, FL). Culture supernatants contained 1–2 μg viral p24 protein/ml and 1×10^6 to 2×10^6 infectious units (IU)/ml. As an estimation, a multiplicity of infection of 1 for $CD4^+$ T cells is equivalent to approximately 1 pg viral p24 per cell (Dewhurst et al., 1987; Shahabuddin et al., 1992). Cells were infected at 1 pg p24 per cell for 1–2 hr at 37°C, followed by washing in phosphate-buffered saline (PBS). As negative control, mock-infected astrocytes were inoculated with the supernatant of nontransfected 293T cells. Infection was monitored by measuring p24 content in cell supernatants and cell lysates by ELISA. Cell lysates were prepared using the instructions and lysis buffer supplied by the manufacturer and standardizing to the number of viable cells.

GFAP Expression and Image Analysis

For immunoperoxidase labeling, sections were incubated overnight in a rabbit GFAP primary antibody (Dako,

Carpinteria, CA; dilution 1:500), followed by incubations in goat antirabbit antibody (Sigma, St. Louis, MO; dilution 1:50) or rabbit peroxidase–antiperoxidase antibody (Sigma; dilution 1:100). Development of the reaction was performed in a solution containing 0.03% diaminobenzidine (DAB; Sigma) plus 0.01% hydrogen peroxide in 0.1 M acetate buffer. After immunostaining, 4′,6′-diamidino-2-phenylindole (DAPI; 0.1 μg/ml; Sigma) was added for 5 min at room temperature to stain all the nuclei of cells in the cultures. Negative controls of the procedure were performed by omitting primary antibody.

The level of expression (area) and intensity of GFAP in infected and mock astrocytes were measured following previously reported protocols. For this goal, sections were observed under identical operating conditions, such as light intensity, wavelength, and gray-scale threshold throughout the experiment. Images were acquired at the same magnification using an Olympus Q5 digital camera connected to a Zeiss Axiophot optical microscope and digitized into gray-level images (Labombarda et al., 2003). All the images obtained were imported into ImageJ (Jacobs and Doering, 2010; version 1.42, National Institutes of Health, http://image-j.nih.gov/ij), and GFAP-positive structures were segmented by defining first a background level in an area of the section with no specific immunoreactivity. This background was determined by examining within the image of the section the distribution (available within the ImageJ software) of pixels by optical density. This distribution has a tail that flattens at the lowest value of optical density (within the tissue). This value was considered the background. The threshold for defining a pixel as "immunoreactive" was set at 20 gray values of optical density over the background (gray levels possible were from 0 to 255, white to black). Thus, a binary image of the area occupied by immunoreactivity was obtained that included all pixels with optical densities higher than 20 gray levels over the background.

Relative optical density (ROD) was obtained after a transformation of mean gray values into ROD by using the formula ROD = log(256/mean gray). For evaluation of the area of immunostained glial cells, the total area of GFAP-positive glial cells was related to the total area of the evaluated field, giving a percentage of immunostained area. At least 10 different fields were measured in each slide. Four slides from each time point PI and controls were obtained. Data are presented as mean values ± SD.

Measurement of Telomerase Activity

Murine astrocytes (1×10^6) were lysed in 200 μl CHAPS buffer and incubated for 30 min on ice. After incubation, lysates were centrifuged at 14,000*g* for 20 min at 4°C. The protein concentrations of the extract were measured using the Bradford assay, followed by dilution of the final concentration of 0.01 μg/μl. Then, telomerase activity (TA) of extracts was measured by quantitative real-time PCR (qPCR) amplification of telomeric repeat fragments, as we have described elsewhere (Reynoso et al., 2010, 2012). The total volume of the reaction mixture was 25 μl per well, containing 12.5 μl 1× SYBR Green Master Mix (Applied Biosystems, Foster City, CA), 0.1 μg TS primer (5′-AATCCGTCGAGCAGAGTT-3′), 0.05 μg ACX primer (5′-GCGCGGCTTACCCTT ACCC TTACCCTAACC-3′), 1 μl RNase-free water, and 10 μl sample. The reaction mixture was incubated for 20 min at 25°C. Then, the PCR was started at 95°C for 15 min, followed by 40-cycle amplification (95°C for 15 sec and 60°C for 60 sec). The assays were performed in triplicate. RNase-free water and heat-inactivated eluates (85°C for 10 min) were used as negative controls. The threshold cycle values (Ct) were determined from semilog amplification plots (log increase in fluorescence vs. cycle number) and compared with standard curves generated from serial dilutions of protein extracts from HeLa cells. TA was quantified and expressed relative to HeLa cells (RTA) using the formula described by Herbert et al. (RTA = 10[Ct sample − Ct positive control]/slope; Hochreiter et al., 2006).

Telomere Length Determination

Telomere length was determined in murine astrocytes by a qPCR as described in detail previously (Cawthon, 2002; Fehrer et al., 2006; Reynoso et al., 2012). In brief, telomere repeat copy numbers (T) and a single copy gene (S; gene 36B4) were amplified using the primers Tel1b 5′-CGGTTTGTTT GGGTTTGGGT TTGGGTTTGGGTTTGGGTT-3′, tel2b 5′-GGCTTGCCTTAC CCTTACCCT TACCCTTACCC TTACCCT-3′, 36B4MF 5′-ACTGGTCTAGGACCCGA GAAG-3, and 36B4MR 5′-TCAATGGTGCCTCTGGAG ATT-3′. Different concentrations of DNA were used for generating a standard curve (2, 20, 200 ng). All samples, for both the T and the S amplifications, were performed in triplicate. The Ct values generated were used to calculate the T/S ratio for each sample using the following equation: $T/S = 2 - \Delta Ct$, where ΔCt = Ct single-copy gene − Ct telomere. T/S ratio is proportional to telomere length (Cawthon, 2009).

Trypan Blue Exclusion Test of Cell Viability

The dye-exclusion test was used to determine the number of viable cells after exposure of murine astrocytes to HIV/VSV. At days 1, 3, 5, 7, and 13, astrocytes were trypsinized, exposed to dye, and then examined visually to determine whether cells take up or exclude dye. The live cells with intact cell membranes exclude trypan blue, whereas dead cells do not.

Statistical Analysis

Data are presented as mean values ± SD, and statistical significance was assessed by one-way ANOVA with Tukey's multiple-comparisons test in GraphPad Prism version 4 (GraphPad Software, San Diego, CA). *P* values were considered to be statistically significant at <0.05. All data shown are representative of at least four independent experiments.

RESULTS

Murine Astrocytes Produce HIV-1 p24 Upon Infection in Culture

HIV-1 pseudotyped with VSV-G causes productive infection in primary murine astrocytes, revealing a peak of p24 production 7 days after infection (Fig. 1). Replication was first detectable at day 3. The exposure of murine astrocytes to HIV-1/VSV allows HIV-1 entry into these cells and increases the susceptibility of astrocytes to HIV-1 infection and replication.

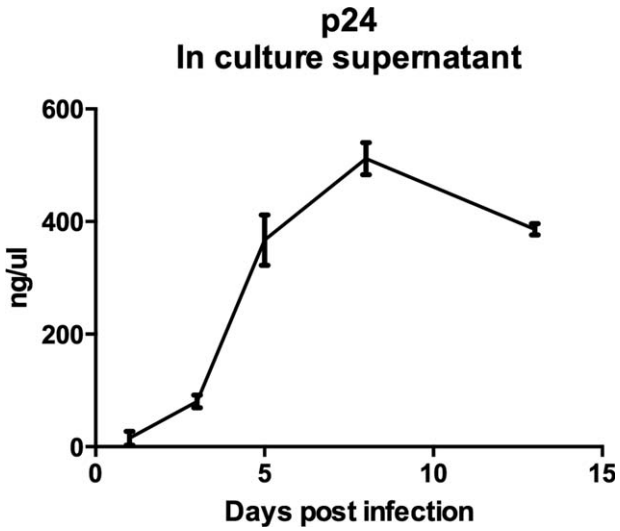

Fig. 1. Infection of primary murine astrocytes monitored by p24 protein expression. Primary murine astrocytes from infected cultures release viral particles. Supernatants of cultivated astrocytes were collected at the indicated times (1, 3, 5, 7, and 13 days postinfection) and titrated for the HIV-1 p24 contents. Results are given as mean values from four independent experiments.

Quantitative Assessment of GFAP Expression

Astrocytes express GFAP; however, the expression of GFAP is increased during activation of astrocytes and astrogliosis. Because astroglial activation is also associated with the HIV exposure, we investigated the role of HIV/VSV infection in the expression of GFAP. After quantitative analyses, GFAP immunolabeling intensity and area for HIV/VSV-infected astrocytes and mock-infected (control) were plotted (Fig. 2). Subsequently, to correlate the expression of GFAP with HIV infection, we analyzed time-dependent expression of GFAP and production of HIV p24 antigen in astrocyte culture supernatants. It is clear that, during the progress of HIV/VSV infection, the expression of GFAP in primary astrocytes is increased, with the maximum observed at the peak of p24 concentration. However, GFAP-positive cells decreased in mock-infected astrocytes, although the number of GFAP-positive cells did not change significantly in pNL43-VSV infected astrocytes. ANOVA revealed significant increases in GFAP immunoreactivity (both labeling intensity and area) 8 days PI in HIV/VSV-infected cells compared with control specimens ($P < 0.0001$). The GFAP expression was also significantly higher 13 days PI but of similar intensity. Hence, the parallel induction of GFAP and HIV p24 production suggests a possible involvement of HIV infection in the increased expression of GFAP in astrocytes.

Measurements of Telomerase Activity (TA)

To test for activation of telomerase, astrocytes were analyzed at 0, 1, 6, 8, and 13 days PI involving previous and subsequent times to the peak of HIV replication, and cell viability was $\geq$95%. TA was measured by qPCR and is displayed relative, or RTA, to that of HeLa cells, which was assumed as 100% (Herbert et al., 2006). Early after HIV infection, a significant ($P < 0.001$) upregulation of TA in infected cells was observed (up to 6 days PI). At days 8 and 13, HIV-infected astrocytes showed TA similar ($P > 0.05$) to that of mock-infected cultures (Fig. 3a). A strong correlation was observed between the augmentation of RTA on astrocytes and the amount of secreted p24 antigen ($R^2 = 0.7423$, Pearson's correlation coefficient -0.8616, $P < 0.0001$; Fig. 3b). Alternatively, in spite expression of both cellular markers, RTA and GFAP, being upregulated after HIV infection, a negative but not significant correlation was observed when considering the values obtained at 1, 6, and 8 dpi ($R^2 = 0.9912$, Pearson's correlation coefficient -0.9956, $P = 0.0599$), given that upregulation of such parameters did not occur simultaneously.

Measurements of Telomere Length (TL)

To test the consequences of telomerase activation in HIV/VSV-infected astrocytes, we analyzed TL in astrocytes in mock-infected vs. infected cells. At 1, 6, 8, and 13 days PI, cells were harvested, genomic DNA was isolated, and TL was studied by qPCR using primers for murine telomere-specific sequences (T) vs. 36B4, a single copy gene (S). The ratio of T/S is thereby proportional to the TL (Cawthon, 2002). At 6 dpi, the telomere to be appeared significantly lengthened ($P < 0.001$) in astrocytes infected with HIV (Fig. 4).

DISCUSSION

In the present study, we have shown that HIV is able to induce activation and dynamic changes in both telomerase activity and TL in cultured murine astrocytes. It is known that cultured astrocytes under basal conditions exhibit a cyclical pattern of telomere lengthening and shortening and are able to divide for much longer periods of time than microglia (Flanary and Streit, 2004). Such replicative capacity is finite after the murine neural stem cell line has differentiated into astrocytes, because telomerase activity is limited and, with each cell division, telomeres shorten progressively (Miura et al., 2001). Moreover, other extra-telomere functions are exerted by this cellular enzyme, including the regulation of calcium distribution (Lin et al., 2007), metabolism (Chung et al., 2005), growth factor secretion (Smith et al., 2003), mitochondrial function (Passos et al., 2007), energy balance (Bagheri et al., 2006), and apoptosis (Massard et al., 2006).

We and others have previously shown that HIV is able to modify the replicative capacity by modulating telomerase activity (Reynoso et al., 2006, 2010, 2012; Franzese et al., 2007; Comandini et al., 2013). The downward modulation of cellular telomerase and telomere erosion provoke progressive tissue atrophy, organ system failure, and impaired tissue injury responses (Jaskelioff et al., 2011). Recently, the measurement of TL from peripheral blood leukocytes was proposed as a

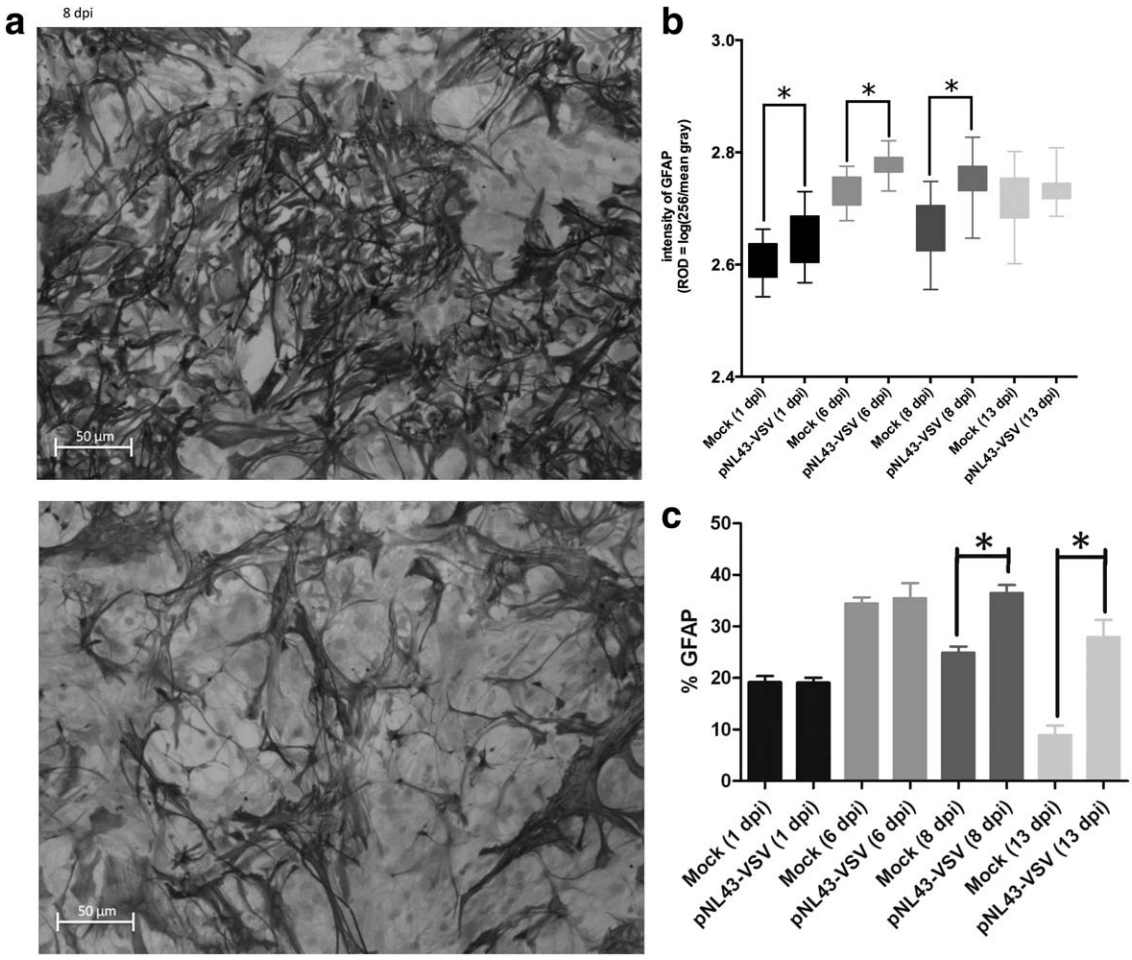

Fig. 2. **a**: Expression of GFAP immunoreactivity in HIV/VSV-infected (top) and mock-infected (lower) astrocytes at 8 days after infection. The expression of astrocytes was detected by immunohistochemical analysis using GFAP, a marker protein for astroglia. ×20. "magnification; scale bar=50 µm" **b:** Relative optical density (ROD) as percentage GFAP immunoreactivity during HIV infection of murine astrocytes. **c:** Percentage of cells expressing GFAP during HIV infection of murine astrocytes. Significant differences ($P < 0.001$) between mock and HIV/VSV-infected astrocytes are indicated by an asterisk.

susceptibility factor in the development of HANDs (Malan-Muller et al., 2013).

Activated astrocytes can exert both protective and detrimental effects on neurons. The contribution of astrocyte activation to HIV-mediated neuropathogenesis also involves astrocyte dysregulation after exposure to HIV particles, viral proteins, cytokines, and other mediators secreted by HIV-infected cells (Borjabad et al., 2010). Studies in vitro indicate that many of these products significantly modulate astrocyte physiology, which in turn can alter essential interactions of astrocytes with other cells in the brain, particularly neurons (Wang et al., 2008). Although HIV–astrocyte interaction studies should ideally be conducted with human cells, primary astrocytes can be obtained only from fetal tissues, but, taking into account the difficulties inherent in obtaining this source from several donors, we decided to use mouse tissues to investigate the impact of HIV infection on the astrocyte telomere/telomerase aging process. To ensure homogeneity of cellular targets, we resorted to brain obtained from a single inbred mouse strain. For this goal, we used murine fetal astrocytes permissive to highly productive HIV infection by HIV NL4-3 strain capable of entry into such CD4-negative cells through pseudotyping with VSV-G envelope protein. Reports from other independent groups indicate that astrocytes, lymphocytes, and macrophages are susceptible to productive infection by pseudotyped HIV as assessed by detection of p24, among other approaches (Canki et al., 2001; Nitkiewicz et al., 2004; Wang et al., 2008).

In the mouse CNS, the developmental schedule of GFAP expression shows its first detection at the end of gestation. Then, after a few days of increment, the expression decreases until reaching a plateau as a marker for brain aging (Riol et al., 1992; Middeldorp and Hol, 2011). Here, after pseudotyped HIV challenge, the murine astrocytes reaching a peak of p24 production 7 days PI, showing a concomitant and significant

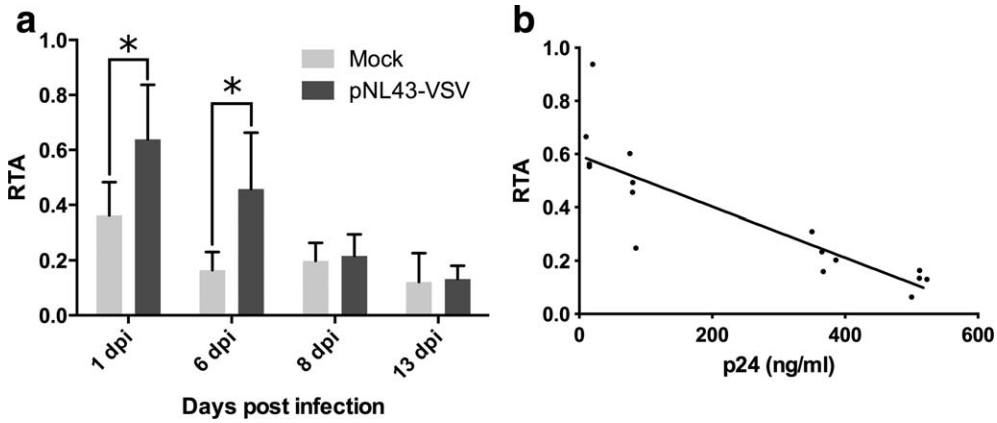

Fig. 3. **a**: Telomerase activity in murine astrocytes harvested at different times (1, 6, 8, and 13 days) after HIV/VSV exposure. Astrocytes were harvested and lysed with CHAPS buffer, and telomerase activity was studied by quantitative real-time PCR and calculated relative to HeLa cells. Significant differences ($P < 0.001$) between mock and HIV/VSV-infected astrocytes are indicated by an asterisk. **b:** Correlation curve between telomerase activity (TA) and levels of p24 antigen (Ag) in culture supernatants. When the levels of antigen p24 production were elevated, the increase in TA was lower ($R^2 = 0.7423$, Pearson's correlation coefficient -0.8616, $P < 0.0001$).

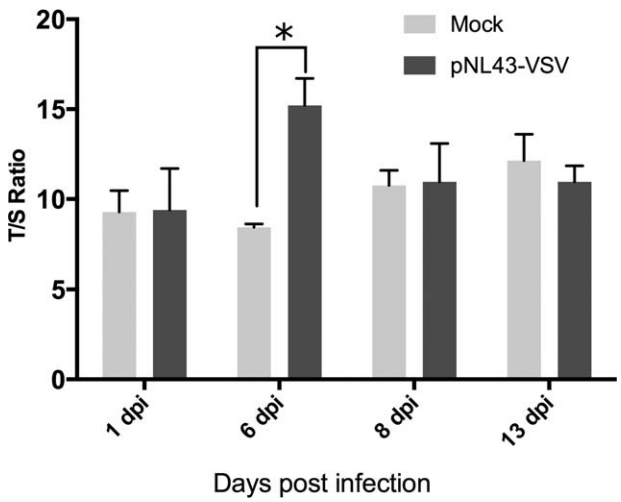

Fig. 4. Telomere length in murine astrocytes harvested at different times (1, 6, 8, and 13 days) after HIV/VSV exposure. Telomere (T) and single-copy gene (S) were amplified by quantitative real-time PCR, with the T/S ratio proportional to telomere length. Significant differences ($P < 0.001$) between mock and HIV/VSV-infected astrocytes are indicated by an asterisk.

activation as revealed by the increased GFAP production. This difference could be related to the action of cytokines such as tumor necrosis factor-α and interleukin-1β produced by HIV-infected astrocytes, by increased intracellular load of oxidatively damaged proteins after viral infection (Ashraf et al., 2011), and by the direct action of HIV proteins such as Tat (Zou et al., 2010; Fan et al., 2011).

In our study, HIV-infected astrocytes showed an early and transient augmentation of telomerase activity after infection, which may contribute to telomere lengthening. Such a phenomenon appears to be inversely correlated with infection progression, and, beyond 3 days PI, when the viral replication reached a peak, both cellular parameters achieved levels similar to those in noninfected astrocytes. This fluctuating telomerase activity appears to be reflected in a cyclical pattern of telomere lengthening. The current results showed that the early increased in telomerase activity in HIV-infected astrocytes might attenuate its proliferation and hence its detrimental effect on neurons (Qu et al., 2011). The enzymatic activity on astrocytes appears to be only transiently upregulated, so such a neuroprotective function might be circumvented according to the HIV infection progress; then, astrocytes became activated, with the reappearance of the detrimental effect that contributes to neuropathogenesis. Taken together, our results suggest that, when HIV induces TA in astrocytes, it might contribute to cellular protection, which could be considered a viral strategy to change its physiology transiently to make it better suited and longer lived.

In spite of HAART benefits, the prevalence of HAND has persisted, and even increased, in older people. Among several hypotheses to explain such a phenomenon, the possible contribution of aging has been advanced. However, it remains unknown whether HIV proteins are involved in the astrocyte aging process, and this deserves further research.

In contrast to our results, Pollicita et al. (2009) reported an oxidative stress-induced apoptosis and telomere shortening. Several factors could explain the discrepancy. U373 is a human immortalized cell line in which TL is maintained as a prerequisite for indefinite cell proliferation, frequently involving high telomerase activity (Bryan and Reddel, 1997). Pollicita et al. used wild-type HIV particles, so cellular changes could be promoted by direct action of viral proteins such as gp120 and

Tat (Price et al., 2005) triggered after their interaction with cellular receptors. By using VSV-G HIV, such interactions are circumvent, because cellular entry occurs by a low-pH-dependent endocytic pathway (Aiken, 1997).

In conclusion, our results shed light on the role of astrocytes in HIV neuropathogenesis. HIV infection promotes two temporally differentiated phenomena. At first, we see telomerase activation and telomere lengthening, which are tightly related to resistance to stress and injury, followed by astrocyte activation, which contributes to neuronal damage. Our data could be useful in understanding the mechanisms involved in HIV-mediated persistence by altering their telomere-related aging processes that could aid in the development of therapeutic modalities, particularly for patients with HAND.

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