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HIV-1 increases extracellular amyloid-beta levels through neprilysin regulation in primary cultures of human astrocytes

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Abstract

Since the success of combined antiretroviral therapy, HIV-1-infected individuals are now living much longer. This increased life expectancy is accompanied by a higher prevalence of HIV-1 associated neurocognitive disorders. Rising too is the incidence in these patients of pathological hallmarks of Alzheimer's disease such as increased deposition of amyloid beta protein (Aβ). Although neurons are major sources of Aβ in the brain, astrocytes are the most numerous glial cells, therefore, even a small level of astrocytic Aβ metabolism could make a significant contribution to brain pathology. Neprilysin (NEP) is a decisive/crucial regulator of Aβ levels. We evaluated the effects of HIV-1 on Aβ deposition and the expression and activity of NEP in primary human astrocytes. Specifically, no differences in intracellular amyloid deposits were found between infected and control cells. However, primary cultures of infected astrocytes showed more extracellular Aβ levels compared to controls. This was accompanied by reduced expression of NEP and to a significant decrease in its activity. These results indicate that the presence of HIV-1 in the brain could contribute to the increase in the total burden of cerebral Aβ.

KEYWORDS
amyloid, extracellular deposits, HIV-1, human, primary cells

1 | INTRODUCTION

Because of the introduction and success of combination antiretroviral therapy (cART), the HIV-1 infection has changed to a chronic disorder. Because of this increased life expectancy (Besson et al., 2001), the HIV-1-infected older population continues to grow. The increased age of infected individuals has a detrimental effect on the progress of the disease, including the development of cognitive dysfunction and neurodegenerative alterations (Brew, Crowe, Landay, Cysique, & Guillemin, 2009).

Enhanced perivascular Aβ accumulation is present in HIV-1 encephalitis and HIV-1 associated dementia brains (Xu & Ikezu, 2009). Several proposed mechanisms describe the increased amyloid beta (Aβ) levels after HIV-1 infection, as the upregulation of its production (Aksenov, Aksenova, Mactutus, & Booze, 2010; Pulliam, 2009), deficient enzymatic degradation (Daily, Nath, & Hersh, 2006; Rempel & Pulliam, 2005), or an increased transport mechanism across the blood-brain barrier (Andras et al., 2010). Furthermore, the local neuroinflammatory responses to the virus could lead to Aβ protein precursor production and susceptibility to its deposition (Esiri, Biddolph, & Morris, 1998; Giunta et al., 2011).

Aβ, mainly peptides of 40 or 42 amino acids, is generated from the sequential proteolytic cleavage from its precursor, the β-amyloid precursor protein (APP). The APP is a type I transmembrane protein

with a single transmembrane domain, a large extracellular ectodomain, and a short cytoplasmic tail. Processing of APP is initiated by β-secretase, resulting in secretion of sAPPβ, and a second membrane-bound C-terminal fragment of APP (βCTF). Subsequent processing of βCTF by γ-secretase generates several Aβ peptides (Hardy & Selkoe, 2002).

The levels of Aβ depend on the balance between its synthesis and degradation rates. One of the mechanisms responsible for Aβ clearance is through enzymatic pathways. Several Aβ-degrading proteases are involved in the regulation of Aβ levels, including neprilysin (NEP), insulin-degrading enzyme, endothelin-converting enzyme, angiotensin-converting enzyme, plasminogen activators, and the matrix metalloproteinases-9 and 2 (MMP-9, MMP-2) (Betts et al., 2008). Among all of these, it has been described that NEP is a major Aβ-degrading enzyme (Farris et al., 2003; Miller et al., 2003), being highly expressed in human astrocytes (Dorfman et al., 2010).

Several data suggest that the reduced NEP levels may lead to the decrease in Aβ degradation, which may cooperate in the development and progression of Alzheimer's disease (AD, Nalivaeva, Fisk, Belyaev, & Turner, 2008).

Diverse studies have demonstrated that HIV-1 Tat protein inhibits NEP activity, and recombinant Tat added directly to brain cultures results in a 125% increase in soluble Aβ (Daily, Nath et al., 2006; Rempel & Pulliam, 2005). In addition, Lan et al. (2011) suggest that Aβ degradation by primary cultured macrophages and microglia is significantly impaired by HIV-1 infection due to a reduction of NEP endopeptidase activity. Besides Tat, gp120 promotes Aβ secretion in primary rat fetal hippocampal cultures (Aksenov et al., 2010) and cause APP accumulation and axonal injury in corpus callosum slices (Zhang, Liu, Katafiasz, Fox, & Xiong, 2011).

As the effects of HIV-1 on NEP expression may represent an important pathological trigger in the AD, the factors affecting NEP expression under these conditions need to be better clarified.

The aim of this study was to investigate a possible regulation of Aβ levels by HIV-1. Moreover, extending previous studies, we targeted the expression of the Aβ-degrading enzyme NEP, in primary human astrocytes before and following HIV-1 infection. Our results indicate that levels of extracellular Aβ deposits in HIV-1-infected cultures of human primary astrocytes are significantly higher than those observed in controls. In parallel, HIV-1 decreased both the expression and the enzymatic activity of NEP. This study suggests that pharmacological maintenance or enhancement of NEP during HIV-1 infection could ameliorate or delay cognitive dysfunction in HIV-1 population.

2 | MATERIALS AND METHODS

2.1 | Cell cultures

Normal human astrocytes (NHA), isolated from the cerebrum of 5-month-old human fetuses were purchased from Cambrex (CC-2565, NHA-Normal Human Astrocytes; Walkersville, MD) and were cultured as described previously (Alvarez, Blanco, Fresno, & Munoz-Fernandez, 2010; Serramia, Munoz-Fernandez, & Alvarez, 2015).

2.2 | Virus stock production

HIV-1 stock production was prepared as described (Alvarez Losada, Canto-Nogues, & Munoz-Fernandez, 2002). All cell types were cultured in the presence of HIV-1_{NL4-3} (200 ng HIV-1 p24Gag/10^6 cells) for 2 hr. After this, cells were washed with fresh medium to remove the viral input and were kept in culture for the indicated times for each condition.

HIV-1 Tat protein (catalog number 2222) was obtained from the NIH AIDS Research and Reference Reagent Program.

2.3 | Assessment of cell viability

The viability of human astrocytes in the presence of Aβ was estimated by determining the activity of mitochondrial dehydrogenases with 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich). Briefly, cells grown into a 96-well plate at a density of 10,000 cells/well were incubated with 5 μM FAM-$Aβ_{1-42}$ for 24 or 48 hr at 37°C. Formazan products were quantified by measuring absorbance at 550 nm with a Dynatech MR5000 plate reader. All points were performed in triplicate.

2.4 | Western blot analysis

Cells were exposed to different stimuli, washed twice with phosphate-buffered saline (PBS) and lysed with 50 mM Tris-HCl pH 7.4, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100 (Sigma, St. Louis, MO) and protease inhibitors (Thermo Scientific, Rockford, IL). Protein contents of the cell lysates were determined by bicinchoninic acid (BCA) protein assay kit (Thermo Scientific). Samples were resolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto polyvinylidene fluoride membranes (Millipore, Bedford, MA) by semidry transference blotting. Membranes were blocked overnight at 4°C using Rotiblock (Roth, Karlsruhe, Germany) before incubation with primary antibodies against NEP (human neprilysin/CD10 antibody; 1 μg/ml; R&D systems, Minneapolis, MN), and β-actin (mouse monoclonal, Sigma). Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Amersham, GE Healthcare, UK; 1:5000) for 1 hr at room temperature. Proteins were detected using the Immun-Star Western C Kit (Bio-Rad Laboratories, Hercules, CA). In all cases, equal amounts of total protein were analyzed across groups. Protein bands were quantified with the Image/J program and normalized to β-actin.

2.5 | FAM-$Aβ_{1-42}$ preparation

FAM-labeled $Aβ_{1-42}$ was purchased from Anaspec (AnaSpec, 34801 Campus Drive, Fremont, CA 94555). The peptide was reconstituted by adding 50 μl of 1% NH_4OH and diluted to 1 mg/ml with DMSO to a final concentration of 0.2 mM.

2.6 | Assessment of Aβ internalization

Cell uptake of FAM-$Aβ_{1-42}$ was measured by plate based assay after incubation of cells at 37°C. At the indicated times, the Aβ-containing

medium was removed and Aβ bound to the cell membrane was quenched with 100 μl 0.2% trypan blue in PBS, pH 4.4 for 1 min. After aspiration, the fluorescence was measured using a Fluorescence Microplate Reader (Synergy 4.0, BioTek Instruments); excitation wavelength was set at 494 nm and the emission wavelength was set at 521 nm. The intensity value was normalized by the number of cells using Hoechst Dye 33342 by the nuclear staining signal (excitation/emission = 350 nm/490 nm).

2.7 | Confocal analysis

Cells were fixed with 2% PFA (Sigma), washed with PBS, and permeabilized with 0.25% Triton X-100 for 5 min at room temperature for membrane permeabilization. Then cells were incubated with 1% BSA (Sigma) for 30 min. When indicated, immunofluorescence staining was performed with Ab-NEP for 30 min at 4°C and the appropriate fluorescent secondary antibody for 30 min at 4°C. After washing, cells were incubated with TRITC-conjugated phalloidin (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, CA) to visualize F-actin cytoskeleton and with DAPI (1 μg/ml; Grupo Taper, Madrid, Spain) to label nuclei. Coverslips were mounted on slides using Dako Fluorescence Mounting Medium (Dako Diagnostics, Barcelona, Spain). Images were obtained using a confocal laser microscopy (LEICA AOBS-TCS-SP2 system, Heidelberg, Germany). Separate images were taken in the corresponding channels, and merged images were composed. Image acquisition and data processing for all samples were performed under the same conditions.

2.8 | Quantitative reverse transcription–polymerase chain reaction

mRNA was extracted using the RNeasy Mini kit and RNase-free DNase set (Qiagen, Hilden, Germany). Only mRNAs showing a poor degradation profile were subjected to Quantitative reverse transcription–polymerase chain reaction (RT-qPCR). Purified mRNAs (1 μg) were used for reverse transcription using the GoScript reverse transcription system (Promega, Madison, WI), and the resulting cDNAs were used for real-time qPCR on a Stratagene Mx3005P block cycler. After normalization to β-actin, the analysis was performed using the $\Delta\Delta C_t$ method (44). The following primer pairs were used. NEP: forward, 5′-CATTGAACTATGGGGGCATC-3′; reverse, 5′-CCTGAAAT TGCCAGGACTGT-3′; β-actin: forward, 5′-AGCCATGTACGTAGCCA TCC-3′; reverse, 5′-CTCTCAGCTGTGGTGGTGAA-3′. Each sample measurement was carried out in triplicate.

2.9 | Cellular NEP activity assay

Treated cells were rinsed twice with ice-cold PBS and lysed in 20 mM Tris-Cl (pH 7.4), 0.5% Triton X-100, 10% sucrose, 1 μg/ml aprotinin, and 10 μM phenylmethanesulfonyl fluoride, all acquired from Sigma-Aldrich (Sigma). The lysates were centrifuged at 15,000 g for 15 min at 4°C, and protein concentrations were measured by the standard BCA assay. Endopeptidase activity was assayed into 96-well plates. In each well, 10 μg lysate and 10 μM fluorogenic peptide Mca-RPPGFSAFK(Dnp)-OH (R&D System, Minneapolis, Minnesota, USA) were mixed in a reaction buffer (100 mM Tris-Cl [pH 7.5], 50 mM NaCl, and 10 μM $ZnCl_2$), and incubated in the dark for 1 hr at 37°C. Thiorphan (2 and 10 μM) (Sigma), a specific NEP inhibitor was added to distinguish specific enzyme activity in the cell lysates. Fluorescent intensity of the cleaved fragments was measured at 320 nm and 405 nm. NEP activity was defined as the activity sensitive to thiorphan inhibition.

2.10 | Flow cytometry

Cells plated at 5×10^5 cells/well in 6-well plates, were infected with HIV-1 (200 ng HIV-1 p24Gag/10^6 cells), and 4 days later, cells were fixed with 2% PFA, permeabilized and incubated with 1% BSA for 30 min. Following blocking, cells were stained with polyclonal goat antihuman NEP (human neprilysin/CD10 antibody) (2.5 μg/10^6 cells) (R&D systems, Minneapolis, MN) for 1 hr and then stained for 30 min with a corresponding fluorescence-conjugated secondary antibody, Alexa Fluor 555 donkey antigoat. After incubation, cells were washed with PBS and collected for analysis by flow cytometry using the EPICS-XL MCL.

2.11 | Statistical analysis

All results are presented as the mean ± standard error of the mean. Differences between groups were analyzed by using a paired Student's *t*-test. Significance levels are indicated as follows: $^{**}p < 0.01$; $^{*}p < 0.05$.

3 | RESULTS

3.1 | Uptake of Aβ by primary human astrocytes

We examined the ability of human astrocytes to internalize amyloid. To determine the optimal concentration of Aβ, we measured the mitochondrial metabolism of the cells treated with the indicated concentrations of Aβ after 24 and 48 hr through MTT assay (Supporting Information Figure S1). In view of the results, the concentration used for the following experiments was 5 μM.

To visualize fluorescent Aβ uptake, cells were incubated with 5 μM FAM-$A\beta_{1-42}$, and, after quenching the extracellular signal, the fluorescence was measured at the indicated time-points. No clear evidence of internalization of the exogenous Aβ was observed compared to the control point without FAM-$A\beta_{1-42}$ (Figure 1a).

To determine accurately the distribution of Aβ, we performed confocal microscopy experiments. After 24 hr of culture with 5 μM FAM-$A\beta_{1-42}$, cells were fixed, permeabilized, and then stained with both TRITC-phalloidin and DAPI for F-actin and nuclei visualization, respectively. In line with previous reports (Nielsen, Veerhuis, Holmqvist, & Janciauskiene, 2009), relatively small FAM-$A\beta_{1-42}$ aggregates were observed within the cell body of astrocytes, whereas the larger and denser aggregates appeared to be preferentially located on the cellular surface. In addition, TRITC-phalloidin staining was consistent between control and treated cells (Figure 1b).

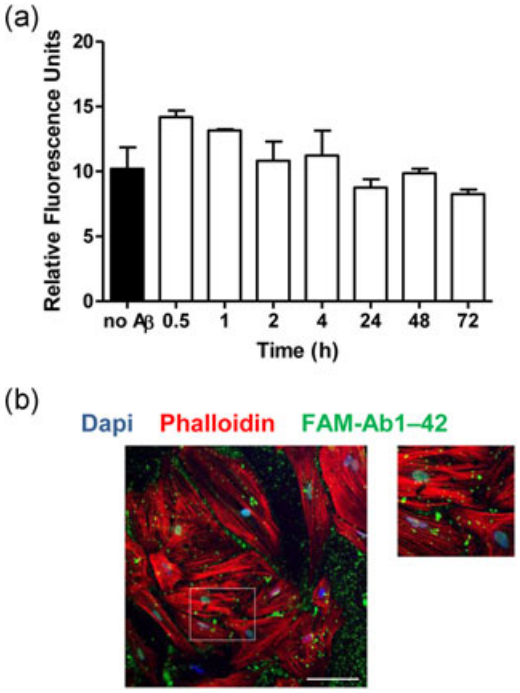

FIGURE 1 Uptake and accumulation of exogenous Aβ in primary human astrocytes. (a) Cells were cultured with 5 μM FAM-$A\beta_{1-42}$, and at the indicated times, fluorescence was measured after quenching extracellular membrane bound Aβ with trypan blue and normalized to Hoechst nuclear staining signal. Aβ internalization over time is shown on the graph. Data represent the ratio Aβ/cell number, referred as relative fluorescence units (RFU), as arithmetic means of at least four independent experiments ± SEM. (b) Merged images of confocal microscopy of cells treated 24 hr with 5 μM FAM-$A\beta_{1-42}$ (green) and stained with TRITC-phalloidin (red) and DAPI (blue). A medial optical section of a representative area is shown. Areas of colocalization are seen in yellow. The zoomed image shows the particle site (boxed region in merged image) together with orthogonal sections. Scale bar, 50 μM [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | HIV-1 increased extracellular levels of Aβ but does not alter Aβ intracellular deposits

It has been reported that recombinant Tat added directly to brain cultures resulted in a 125% increase in soluble Aβ (Daily et al., 2006). However, in our model we cannot detect any alteration in Aβ levels following exposure of Tat (data not shown), and for this, we decided to examine the effect of the complete HIV-1 virus. So, cells were infected with HIV-1 (200 ng HIV-1 p24Gag/10^6 cells) for 4 days and subsequently incubated with FAM-$A\beta_{1-42}$ (5 μM) for additional 48 hr. HIV-1 did not alter the levels of intracellular Aβ (Figure 2a). However, extracellular deposits of Aβ increased almost 3-fold in HIV-1-infected astrocytes compared to controls (Figure 2b) ($^*p < 0.05$). Confocal analysis validated the results indicating the extracellular accumulation of Aβ only in infected cultures (Figure 2c).

To understand the capacity to internalize Aβ of nonbrain cells, we performed the same experiments in HeLa cells. Unexpectedly, an important uptake of Aβ was detected in HeLa cells compared to primary human astrocytes (Supporting Information Figure S2). Albeit a quick uptake even at 2 hr of treatment, the levels of intracellular FAM-$A\beta_{1-42}$ dropped with the time in contrast with the observed for astrocytes. These observations are in contrast with previously published findings (Knauer, Soreghan, Burdick, Kosmoski, & Glabe, 1992) suggesting no cellular specificity in the uptake of FAM-$A\beta_{1-42}$.

3.3 | HIV-1 reduced NEP expression

Based on previously described regulation of NEP by HIV-1 in other cells (Aksenov et al., 2010; Lan et al., 2011), we initially hypothesized that NEP would be suppressed in primary human astrocytes by HIV-1 being responsible, at least in part, for raised levels of extracellular Aβ. To specifically address this, we performed qPCR analysis of NEP expression and although we found that HIV-1 showed a trend to suppress NEP mRNA without significance (Figure 3a). In addition, immunoblot analysis revealed that HIV-1 induced a statistically significant reduction in NEP protein expression level ($^{**}p < 0.01$) (Figure 3b) meanwhile levels of β-actin were similar in all samples. This observation was further confirmed by immunocytochemistry, where noninfected (control) cells were stained with a mouse-anti-NEP monoclonal antibody (red) with more intensity than infected astrocytes Nuclei were counterstained with DAPI (blue; Figure 3c).

Because NEP can be intracellular as well as an exposed enzyme located on the surface of the cell membranes, we examined specifically whether HIV-1 could alter surface expression of NEP. A flow cytometry analysis revealed that NEP-positive cells were approximately 10% of the total population, and less than 5% presented the protein on the cell surface. After infection, no significant changes were observed in the surface and total NEP-positive cells (Figure 3d). Because the great majority of changes for NEP levels were observed at the mean fluorescence intensity (MFI), we decided to quantify the integrated MFI (iMFI, % of positive cells *MFI). Cell surface NEP MFI levels in HIV-1-infected primary astrocytes were significantly lower in comparison to controls ($^{**}p < 0.01$; Figure 3e).

3.4 | NEP activity was inhibited by HIV-1 in primary human astrocytes

Because extracellular levels of Aβ have been previously related to NEP endopeptidase activity, we wondered whether HIV-1 directly could inhibit NEP activity in vitro. Thus, HIV-1-infected cells (4 dpi) were incubated with the enzyme-specific fluorogenic substrate Mca-RPPGFSAFK(Dnp)-OH, as well as with a NEP inhibitor, thiorphan at the concentrations of 2 and 10 μM. NEP endopeptidase activity was decreased by HIV-1 infection at 4 dpi (Figure 4a), reaching statistically significance after the subtraction of fluorescence in the presence of thiorphan 10 μM (Figure 4b).

4 | DISCUSSION

Antiretroviral therapy has led to long-term survival for HIV-1-infected individuals. Thus, the number of infected persons has increased along with the proportion of those who are considered "older" (over the age

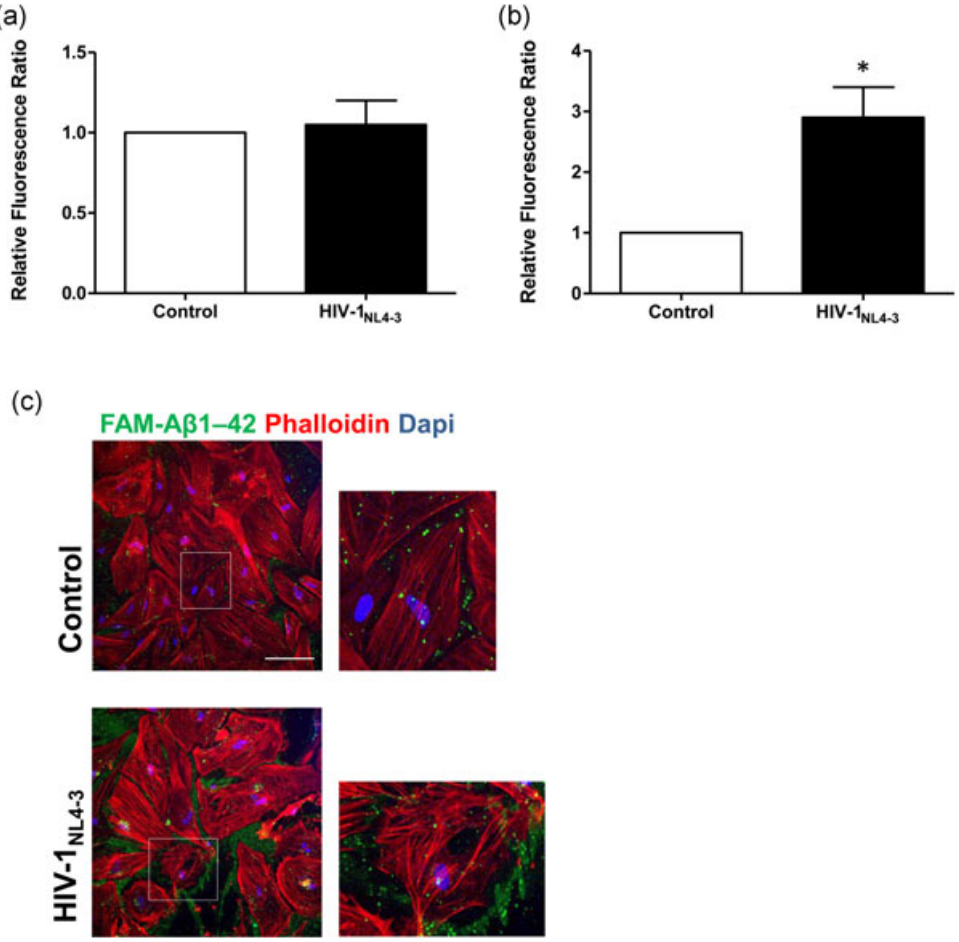

FIGURE 2 HIV-1 leads to increased extracellular deposits of Aβ in primary cultures of astrocytes. Cells were infected (black bars) or not (white bars) with 200 ng HIV-1 p24Gag/10^6 cells for 4 days, followed by incubation with 5 μM FAM-$Aβ_{1-42}$ for additional 48 hr. Intracellular (a) or extracellular (b) Aβ levels were calculated as Aβ fluorescence measured with or without trypan blue (intracellular and total Aβ respectively; extracellular Aβ = total − intracellular). Data represent the relative fluorescence ratio (RFR; ratio Aβ/cell number, referred to nontreated control cells), as arithmetic means of three independent experiments ± SEM. (*$p < 0.05$). (c) Confocal microscopy of control (left panel), and HIV-1-infected cells (right panel) exposed to 5 μM FAM-$Aβ_{1-42}$ for 48 hr. Visualization of the F-actin and the nuclei stained with TRITC-phalloidin (red) and DAPI (blue) respectively. A medial optical section of a representative area is shown. Areas of colocalization are seen in yellow. The zoomed images show the deposits of FAM-$Aβ_{1-42}$ (boxed region in left images). Scale bar, 50 μM [Color figure can be viewed at wileyonlinelibrary.com]

of 50; Goodkin et al., 2001). While the incidence of HIV Associated Neurocognitive Disorders (HAND) has declined since the introduction of cART, its prevalence appears to be on the rise (Dore, McDonald, Li, Kaldor, & Brew, 2003; McArthur, 2004). In addition, in older HIV-1-infected individuals is very common the incidence of AD-like pathology (Achim, Adame, Dumaop, Everall, & Masliah, 2009), showing the presence of extracellular amyloid deposits similar to those detected in patients with AD (Rempel & Pulliam, 2005). However, the Aβ deposition presents different characteristics in AD and HIV-1 brains. Therefore, while extracellular amyloid accumulation is the major amyloid pathology in AD, intraneuronal amyloid deposits are significantly higher than extracellular ones in HAND (Xu & Ikezu, 2009). We did not find these differences in intracellular amyloid levels between infected and control cells.

Compared to microglial cells, our understanding of human astrocyte-mediated Aβ internalization and consequences is limited. Although several mechanisms have been suggested to explain the internalization and degradation of the peptide in these cells (Koistinaho et al., 2004; Saavedra, Mohamed, Ma, Kar, & de Chaves, 2007; Wyss-Coray et al., 2003), the exact way remains to be clarified. It is interesting to remark that our work is performed exclusively using primary cultures of astrocytes; this is an important issue considering that most of previously reported investigations in this line have been done in mouse models (Pihlaja et al., 2008; Wyss-Coray et al., 2003).

Although multiple studies have shown how HIV-1 infection may alter Aβ degradation rates resulting in increased extracellular deposits, the mechanisms involved are not completely understood. It has been previously shown that cART resistance, chronic HIV-1 infection, drug toxicity and reduced clearance of misfolded proteins might play a role (Hult, Chana, Masliah, & Everall, 2008). The localization of NEP on the plasma membrane with its catalytic site exposed to the extracellular space makes this peptidase a prime candidate responsible for the increase in Aβ accumulation induced by

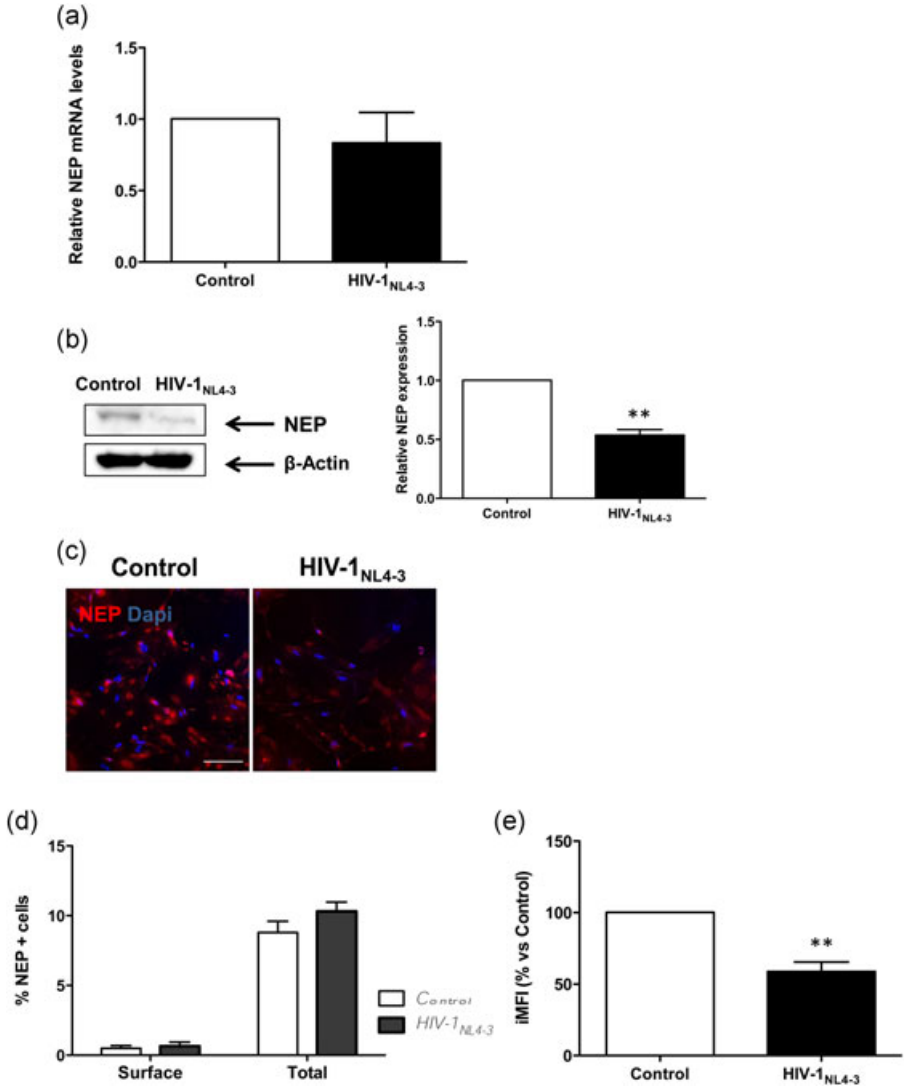

FIGURE 3 HIV-1 downregulates NEP expression in primary human astrocytes. Cells were infected with 200 ng HIV-1 p24Gag/10^6 cells for 4 days. (a) NEP mRNA levels were quantified by qPCR. Fold increase was calculated as a ratio of NEP mRNA in HIV-1 infected compared to control cells. Transcript levels were normalized to β-actin levels ($n = 3$) (b) Western blot analysis of NEP expression in cell lysates from control and HIV-1-infected cells (left panel). The relative signal intensity (NEP/β-Actin) for each sample was measured, and normalized values are shown in the graph (right panel) (**$p < 0.01$). A representative blot is shown, and data are expressed as means ± SEM from two independent experiments. (c) Merged images of confocal microscopy of control (left panel) and HIV-1-infected cells (right panel stained with anti-NEP (Alexa-555, red) and DAPI (blue)). Bar scale: 10 μM. (d) Flow cytometry results of total NEP expression in control (white bars) and HIV-1-infected cells (black bars), ($n = 3$) (e) Quantification of the integrated mean fluorescence intensity (iMFI) levels of surface-expressed NEP in control and HIV-1-infected cells. The results represent the percentage of iMFI relative to that of control cells normalized to 100%. (**$p < 0.01$), ($n = 3$) [Color figure can be viewed at wileyonlinelibrary.com]

HIV-1. Numerous studies have involved NEP as a rate-limiting Aβ-degrading enzyme in the brain, including ectopic NEP expression in primary neurons (Hama et al., 2001), knockout studies in mice (Iwata et al., 2001), and chronic transgenic overexpression studies (Leissring et al., 2003). Mothapo et al have described detectable plasma $A\beta_{1\text{-}42}$ levels in HIV-related cognitive impairments (67%), when compared to HIV without cognitive alteration (29%) and controls (10%), presenting it as an interesting biomarker (Mothapo et al., 2015). Aβ degradation by primary cultured macrophages and microglia is significantly impaired by HIV-1 due to a reduction of NEP endopeptidase activity (Lan, Xu et al., 2011), and more recently, it has been determined the relation between the expression of Nef and Aβ peptides (Khan et al., 2016).

In summary, we demonstrate that there are no differences in intracellular amyloid levels between infected and control astrocytes. However, extracellular Aβ accumulation is significantly increased by HIV-1. This is accompanied by a significant reduction of NEP expression and a malfunction of NEP demonstrated by the impaired endopeptidase activity. Our experiments show that it is possible that the processes limiting the extracellular accumulation

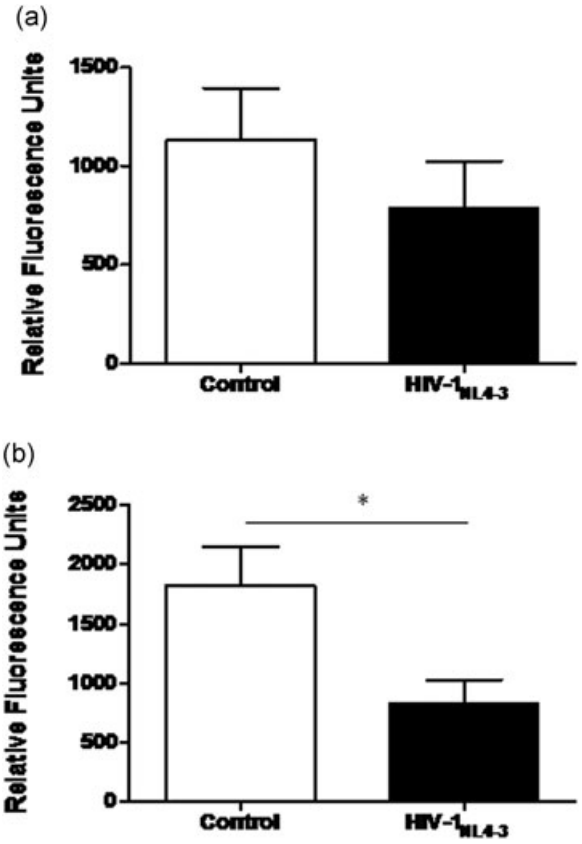

FIGURE 4 HIV-1 decreases enzymatic activity of NEP in primary human astrocytes. NEP activity was measured in cell lysates using a fluorogenic peptide substrate. The specific NEP activity was calculated by subtracting residual fluorescence after incubation with the selective NEP inhibitor, thiorphan 2 μM (a) and 10 μM (b). The histograms represent the NEP activity referred as relative fluorescence units (RFU), as arithmetic means of six independent experiments ± SEM (*$p < 0.05$)

of Aβ could be impaired in the presence of HIV-1. We believe that exploring the molecular pathways responsible for this, and the mechanisms associated will suggest new therapeutic strategies for the effective clearance of Aβ in the brain of HIV-1-infected individuals.

Several studies have attempted to address certain aspects of this topic, but to our knowledge, there are few data integrating all these questions, that are HIV-1, primary human astrocytes, and amyloid.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

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