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Immuno-Neuropathogenesis of HIV-1 Clade B and C: Role of Redox Expression and Thiol Modification

Thangavel Samikkannu, **Kurapati V.K. Rao**, **Sudhessh Pilakka Kanthikeel**, **Venkata Subba Rao Atluri**, **Marisela Agudelo**, **Upal Roy**, and **Madhavan P.N. Nair***
Department of Immunology, Institute of NeuroImmune Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, Florida 33199

Abstract

Previous studies have shown that infection with HIV-1 clade B and clade C differentially contributes to the neuropathogenesis and development of HIV-associated neurocognitive disorders (HAND). The low molecular weight tripeptide glutathione (GSH) alters the redox balance and leads to the generation of reactive oxygen species (ROS), which play a significant role in the neuropathogenesis of HAND. We hypothesized that the HIV-1 clade B and clade C viruses and the respective Tat proteins exert differential effects on monocyte (MC)-derived immature dendritic cells (IDC) and neuroblastoma cells (SK-N-MC) by redox activation, which leads to immuno-neuropathogenesis. The GSH/GSSG ratio and mRNA expression levels and protein modification of glutathione synthetase (GSS), glutathione peroxidase 1 (GPx1), superoxide dismutase 1 (SOD1) and catalase (CAT) were analyzed in IDC infected with HIV-1 clade B or clade C as well as in cells treated with the respective Tat proteins. The results indicated that HIV-1 clade B virus and its Tat protein significantly increased the production of reactive oxygen species (ROS) and reduced the GSH/GSSG ratio and subsequent down-regulation of gene expression and protein modification of GSS, GPx1, SOD1 and CAT than infection with the clade C virus or treatment with the clade C Tat protein. Thus, our studies demonstrate that HIV-1 clade B and C exert differential effects of redox expression and thiol modification. HIV-1 clade B potentially induces oxidative stress, leading to more immuno-neuropathogenesis than infection with HIV-1 clade C.

Keywords

HIV-1 clade B and C; dendritic cells; neuron; oxidative stress and glutathione

*Corresponding Author: Madhavan P.N. Nair, Ph.D., Department of Immunology, Professor and Chair of Immunology, Institute of NeuroImmune Pharmacology, Herbert Wertheim College of Medicine, ACH-1# 308, Florida International University, Modesto A. Maidique Campus (MMC), 11200 S.W. 8th Street, Miami, FL-33199, Tel: 305-348-1493, Fax: 305-348-1109, nairm@fiu.edu.

Introduction

Human immunodeficiency virus type-1 (HIV-1) displays extraordinary genetic variation in its global distribution. It is classified into four groups (M, N, O and P) and nine different subtypes or clades (A-K) based on phylogenetic analysis [1]. A large majority (>86%) of circulating HIV-1 are B and C clade, and clade C is responsible for more than 56 % of HIV-1 infections. These subgroups M, N, O and P differ from amino acids in 30–40 % and subtypes or clades (A-K) 5–20% respectively [2]. The predominant subtype of HIV-1 clade B infections occurs in North America, Western Europe, and Australia. Conversely, HIV-1 clade C is found in Africa, Latin America, and Asia) [3–5].

HIV-1 directly affects the immune function causing a deficiency in antigen presentation. This is manifested by a dysregulation of inflammatory cytokines, chemokines, and other factors such as oxidative stress-induced reactive oxygen species and reactive nitrogen species [6–8]. *In vitro* and *in vivo* evidence shows that HIV infection affects peripheral cells such as MC and DC as well as the central nervous system (CNS), leading to immune dysfunction [9–11]. These immune dysfunctions and pathogenic mechanisms tentatively imbued with the ability to enter the CNS can then induce neuropathogenesis. Studies have shown that HIV-1 directly and indirectly affects the CNS, causing neurological impairments that are manifested by cognitive, behavioral, and motor abnormalities due to the massive death of neurons in all regions of the brain [12]. The HIV Tat protein is known to cause cellular oxidative stress and progressively affects the CNS. It is also essential for viral replication and disease progression-induced neuronal impairments [13, 14] and stimulates nitric oxide synthase [15] as well as other toxic factors. Previous studies have shown that Tat is released extracellularly by HIV-1-infected lymphocytes and microglial cells [16]. Interestingly, Tat mRNA expression is increased during HIV brain dementia [17]. In astrocytes, Tat induces oxidative stress, affecting mitochondrial function and leading to cell death [18]. Tat may also interact with cell surface receptors, leading to the activation of intracellular signaling pathways [19]. Previous studies have suggested that the biological properties of HIV clades influence disease progression, transmission efficiency and dissemination [20]. The amino acid divergence of Tat among HIV-1 clades may influence its binding and transactivation functions [21] and is also associated with the varying degrees of associated neurological problems [12]. Recently, Mishra et al. reported that alterations in the dicysteine motif at position C30C31 in the clade C Tat protein likely alter its functional properties [22].

Recent studies have suggested that HIV-dementia is associated with increased oxidative stress and altered lipid peroxidation in the brain [23]. The oxidative stress-induced free radicals H_2O_2 and O_2- cause cellular damage in many diseases, including HIV/AIDS [24, 25]. Glutathione (GSH) is one of the main players in intracellular antioxidant defense mechanisms, and low levels of GSH have been associated with impaired immune responses and neuronal dysfunction [26]. The reduced level of GSH in HIV-positive individuals results in an increased production of H_2O_2 and O_2- [27], leading to AIDS dementia complex (ADC) [28–29]. It has been shown that sequence variations in HIV-1 viral proteins lead to differential expression of dementia and neurocognitive disorders [20, 12]. However, the underlying mechanisms of how immune dysfunction in immature dendritic cells (IDC) leads

to neuronal cell loss and ultimately HIV-associated neurocognitive disorders (HAND) are not well understood. Therefore, we investigated the mechanism of differential induction of oxidative stress by HIV-1 clades B and C by assessing alterations in redox expression and thiol modification in IDC and SK-N-MC cells.

We demonstrated that HIV-1 clade B virus and the clade B Tat protein induced oxidative stress and potentiated redox-induced gene expression and protein modification of GSS, GPx1, SOD1 and catalase in IDC and neuronal cells; the effects were significantly different than those associated with clade C infection or clade C Tat protein.

Materials and Methods

HIV-1 clade B and C viruses and Tat recombinant proteins

HIV-1 clade B (Bal strain) and clade C (CN54 strain) viruses and the HIV-1 clade B Tat protein were obtained from the NIH AIDS Research and Reference Reagent Program (catalog numbers 510, 4164 and 2222, respectively). The HIV-1 clade C Tat was obtained from Diatheva (Fano (PU), Italy). The purity and functional properties of the recombinant Tat proteins were confirmed by a transactivation assay.

Isolation of monocyte-derived immature dendritic cells (IDC)

IDC were prepared from peripheral blood mononuclear cells (PBMC) as described by Nair et al. [30]. Briefly, PBMC were separated on a density gradient and adhered to plastic culture plates containing media plus serum. Nonadherent cells were removed after incubation for 2 h at 37°C, and adherent cell monocytes (MC) were harvested and washed with PBS/1% FCS. MC were grown for 5–7 days in RPMI culture medium containing 500 U/mL of GM-CSF and 500 U/mL of IL-4 to generate IDC. Subsequently, the IDC were stained with the surface markers CD80, CD86, CD40 and CD11c and analyzed by flow cytometry.

Neuronal Cell Culture

The neuroblastoma cell line SK-N-MC was purchased from ATCC (Manassas, VA), and the cells were cultured in Eagle's minimum essential medium (MEM) supplemented with fetal bovine serum to a final concentration of 10% and 1% antibiotic/antimycotic solution (Sigma-Aldrich, St. Louis, MO).

HIV clade B and C virus infection of IDC

IDC (1×10^6 cells/ml) were treated with polybrene (4 μg/ml) for 5 hours and then infected with HIV-1 clade B or clade C at 20 ng/10^6 or $TCID_{50}$ (the dose that produces approximately the same levels of P24 antigen in culture) at 37°C. After 18 hours, the cells were washed with PBS, and the HIV infections were maintained for 15 days. Every third day, half of the medium was replaced with fresh medium, and the supernatant obtained from the removed medium was used for the p24 antigen estimation using an ELISA kit. Uninfected control cells were included in all of the experiments.

HIV-1 clade B and C Tat protein Treatment of IDC and SK-N-MC

IDC and SK-N-MC (1×10^6cells/ml) were treated with either HIV clade B Tat or clade C Tat protein using an optimized concentration of 50 ng/ml for 24 hr treatment. The untreated cells served as the control.

ELISA - p24 measurements

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Zeptometrix, Buffalo, NY, USA) was used to quantitate the amount of p24 in the culture supernatants.

Determination of reactive oxygen species (ROS) production and intracellular thiol conent

The HIV-1-induced-ROS production and intracellular thiol levels were analyzed by flow cytometry in a FACScaliber (BD Bioscience, San Jose, CA). Briefly, IDC (5×10^5) were infected with 20 ng HIV-1 clade B or clade C for 6 days. At the end of the time period, either 10 μM 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) or amplex red and 50 μM monobromobimane (mBBr) (Invitrogen, Carlsbad, CA) was added directly to the medium and the cells were incubated at 37°C for 30 min. The cells were then washed with PBS, and ROS production and intra cellular thiol levels were analyzed.

Effect of HIV-1 clade B and C on DC-SIGN Expression in IDC

The HIV-1-induced expression of the DC-specific intercellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin (DC-SIGN) was analyzed by flow cytometry in a FACSCaliber (BD Bioscience, San Jose, CA). Briefly, IDC (5×10^5) were infected with either HIV clade B or C (20 ng/10^6 cells) for 6 days. Then, the cells were washed, and surface staining was performed using FITC-conjugated anti-DC-SIGN antibody (Invitrogen, Carlsbad, CA). The cells were then washed with PBS, and DC-SIGN expression levels were analyzed.

RNA extraction and real-time quantitative PCR (qPCR)

Total RNA from 1×10^6 IDC and cultured SK-N-MC cells was extracted using a Qiagen kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The total RNA (5 μg) was used for the synthesis of the first strand of cDNA. The amplification of cDNA was performed using primers specific for the LTR R-U5 domain (Sigma, St. Louis, MO), GSS (Assay ID Hs00609286), GSR (Hs00167317), SOD1 (Hs00166575) catalase (Hs00156308), and β-actin (Hs99999903) (Applied Biosystems, Foster City, CA). β-actin served as an internal control. The relative abundance of each mRNA species was assessed using brilliant Q-PCR master mix from Stratagene using Mx3000P instrument which detects and plots the increase in fluorescence versus PCR cycle number to produce a continuous measure of PCR amplification. The relative mRNA species expression was quantitated, and the mean fold change in expression of the target gene was calculated using the comparative CT method (transcript accumulation index, TAI = $2^{-\Delta\Delta CT}$). All of the data were normalized for the quantity of RNA input by performing measurements on an endogenous reference gene, β-actin. In addition, the results obtained with RNA from treated samples were normalized to the results obtained with RNA from the control, untreated sample [31].

Western blot

To assess protein expression, IDC and SK-N-MC cells (1×10^6) were treated with HIV-1 clade B or clade C Tat proteins (50 ng/ml), for 24 h at 37° C. After incubation, the cells were washed with PBS and lysed using lysis buffer (Pierce, Rockford, IL) with a 1x complete cocktail of protease inhibitors. The extracts were centrifuged at 14,000 g for 5 min at 4°C, and the total amount of cellular protein was determined by a Bio-Rad protein determination assay (Bio-Rad, Hercules, CA, USA). Equal amounts of proteins were resolved by 4–15% polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. After blocking, the membrane was probed with primary rabbit polyclonal GSS and GPx1 and mouse monoclonal SOD1 antibody (Chemicon International, Temecula, CA); followed by secondary goat antirabbit IgG antibody and goat antimouse IgG antibody (Santa Cruz Biotechnology, Paso Robles, CA). The immunoreactive bands were visualized using a chemiluminescence western blotting system according to the manufacturer's instructions (Amersham. Piscataway, NJ).

Determination of GSH/GSSG

The cellular GSH/GSSG ratio was determined by a colorimetric assay (Catalog number GT 35, Oxford Biomedical Research, Inc. Oxford, MI). Briefly, IDC (1×10^6) were treated with either 50 ng HIV-1 clade B Tat or clade C Tat for 24 h, and the cells were washed with PBS and then resuspended in 1 ml of a homogenization buffer containing HEPES buffer (pH 7.4) with 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH2PO4 and 0.6 mM MgSO4 and homogenized with 10 strokes using a chilled Dounce homogenizer. The homogenate was centrifuged at 14,000 rpm for 10 min at 4°C, and the supernatant was collected. The GSH/GSSG ratio was determined according to the manufacturer's instructions and expressed as GSH equivalents using a standard curve.

Statistical analysis

The results presented in this study are representative of three or more independent experiments performed in triplicate. Statistical significance was determined by ANOVA, Student's t-test or unpaired observations using Graph Pad Prism Software, Inc. (La Jolla, CA). The values were presented as the mean ± SD, and a p-value of ≤ 0.05 was considered significant.

Results

HIV-1 clade B and C infection induced oxidative stress and redox-dependent gene expression in IDC

It is known that HIV infection and Tat protein induce ROS production and alter redox regulation, leading to apoptosis and peroxidative damage [17, 24, 25 32]. However, the effect of specific HIV clades on redox-dependent gene expression has not yet been studied. Therefore, we examined whether HIV clade B and C viruses induce similar or distinct mechanisms of redox-dependent expression in IDC. The results demonstrated that after 6 days of either clade B or C virus infection, oxidative stress was significantly increased and redox-dependent expression was subsequently inhibited in IDC. Figure 1 shows the effects

of clade B and C viruses on the level of p24 antigen using 20ng virus (A) or $TCID_{50}$ (B) as well as the effects of the viruses on LTR-R/U5 (C) and GSS (D) gene expression. These results demonstrate that compared with HIV clade C infection, HIV clade B infection results in a significant increase in p24 antigen secretion and LTR-R/U5 expression ($p<0.01$) and a subsequent inhibition of GSS gene expression ($p<0.004$). Figure 2 shows that infection with HIV clade B virus induced oxidative stress in IDC, leading to increased ROS production by DCFH-DA (A), ($p<0.02$) amplex red (B) ($p<0.02$) and subsequently depleting intracellular thiol (C) ($p<0.004$) compared to clade C infection. These results suggest that HIV-1 clade B and clade C differentially regulate thiol protein and redox-dependent gene expression; clade B has a more potent effect than clade C.

HIV clade B and C infection induced DC-SIGN expression in IDC

DC-SIGN, also referred to as CD209, plays a key-role in the dissemination of HIV infection [33, 34]. However, the ability of specific HIV clades to induce DC-SIGN expression has not yet been studied. Therefore, we determined the effect of HIV clade B and C viruses on DC-SIGN expression in IDC. Fig 3 demonstrates that HIV clade B infection showed a significant increase in DC-SIGN expression at 6 days compared with HIV clade C infection. These observations confirmed that clade B infection potentiates and activates DC-SIGN-mediated pathogenesis compared to clade C infection.

HIV-1 clade B and clade C Tat induced redox-dependent gene expression in IDC

Next, we tested whether exposure to HIV-1 clade B or C Tat proteins induces oxidative stress and redox-dependent gene expression similar to HIV viral infection. IDC treated with Clade B Tat for 24 h showed a greater inhibition of GSS ($p< 0.01$) (A), GPx1 ($p< 0.009$) (B), SOD1 ($p< 0.03$) (C) and CAT ($p< 0.003$) (D) gene expression compared with IDC exposed to clade C Tat (Figure 4). These observations confirmed that clade B and C differentially regulate redox-dependent gene expression.

Effect of clade B and C Tat on GSH/GSSG ratio and intracellular thiol

The GSH/GSSG ratio plays an important role in oxidative stress mechanisms and the maintenance of redox balance. We investigated the capacity of HIV-1 clade B and clade C Tat to influence this ratio in IDC. Twenty-four hours after treatment, a lower GSH/GSSG ratio was detected in clade B Tat-treated IDC than in clade C Tat-treated cells ($p< 0.01$) compared to control. However, clade C is not significantly impacted compared to control. (Figure 5A), indicating that clade B Tat has a more pronounced effect on the GSH/GSSG ratio than clade C Tat. We also analyzed the level of intracellular thiol in IDC treated with clade B Tat or clade C Tat. Fig 5B shows that IDC treated with clade B Tat showed significantly reduced thiol levels ($p<0.02$) than IDC treated with clade C Tat or control. These results are consistent with the observed effects of HIV-1 clades B and C on ROS production and GSS gene expression (Fig 1 and 2).

Effect of clade B and C Tat on redox-induced protein modification

We also examined whether clade B and clade C Tat differentially modulate thiol oxidation and redox enzymes leading to protein modification. Figure 6 demonstrates that clade B Tat-

treated-IDC showed a significant decrease in GSS (A), GPx1 (B) and SOD1 (C) protein modification at 24 hr of treatment compared with untreated cells, whereas no changes were detected in clade C Tat-treated IDC. Taken together, these findings suggest an important role of redox expression by HIV-1 clade B and clade C infection lead distinct mechanism of immune function.

HIV-1 clade B- and clade C-induced thiol modification and redox expression

HIV infection affects immune function and ultimately impact CNS functions [35, 36]. DCs are one of major reservoirs of connection between immune and CNS function. Therefore, we examined the effect of redox expression by clade B and clade C Tat on SK-N-MC neuroblastoma cells. However, the structural and genome of Tat sequence changes the motif and alters the functional property [22], which may impact distinct functional effects. Figure 7 shows a comparison of the clade B and clade C Tat primary structural sequences (A), the increased production of ROS (B) and subsequent reduction in GSS gene expression (Figure 7C) and protein modification (D) in SK-N-MC treated with clade B Tat compared with cells treated with clade C Tat. This study suggests that immune dysfunction subsequently leads to impaired neuronal function, and this effect might be mediated differentially by HIV-1 clade B and C infections.

Discussion

The present study provides new insights into the differential impact of HIV-1 clade B and C infection on oxidative stress induced redox-expression and thiol modification in IDC and SK-N-MC. Here, we demonstrated how the clade-specific viruses and Tat proteins modulate the efficacy of HIV-1 infection through redox balance and antioxidant status. This is the first report to demonstrate that clade B and clade C infections differentially induce redox expression and thiol modification. In addition, our results suggest that the HIV-1 clade B virus- and Tat protein-induced redox regulation and thiol modification significantly decrease GSH levels and potentiate more immune-neuropathogenic dysfunction compared with clade C virus and Tat protein.

Previous studies have shown that HIV-1 Tat protein is capable of facilitating HIV infection [37], viral replication and disease progression [38]. HIV-Tat treatment of various CNS cells induces oxidative stress and alters the mitochondrial membrane function, possibly leading to brain dementia and neuronal cell death [39, 23]. It has been reported that oxidative stress is coupled with different genetic or environmental insults to affect different CNS regions [40] and trigger astrocytes and microglia [41, 42). Studies have shown that HIV-1 Tat-induced oxidative stress leads to decreased GSH levels in DC *in vitro* and *in vivo*, which could affect antigen-presenting cells (APC) [43] as well as CNS function. In this regard, concomitant low levels of SOD could lead to elevated ROS production and lipid peroxidase levels (LP) [44, 45]. Previous studies have demonstrated that HIV-1 clade B significantly potentiates the inhibition of antigen processing and presentation in IDC [46]. However, DC-SIGN is the major player in the DC function and directly or indirectly affects the signaling machinery via ROS. HIV infection is known to cause DC-mediated immune function [30]. Recently, Li et al. suggested the potential role of DC-SIGN as a receptor for DNA and protein and

proposed that it induces tolerogenicity in DC through DC-SIGN-mediated negative signals [47]. Studies have shown that changes in cellular redox status induce oxidative stress in neuronal cells, which results in the depletion of GSH [48] and increased oxidized protein carbonyls [49] and reactive oxygen intermediates in CSF in patients with severe dementia [50]. This suggested that redox state imbalance and GSH activity is associated with neuronal dysfunction. However, there are no reports on the cellular and molecular mechanism of redox-induced gene expression initiated by HIV-1 clade B and C.

In the present study, we have shown for the first time that clade B infection increases ROS production and decreases intracellular thiol and GSS gene expression compared with clade C infection (Figs. 1 and 2). HIV-1 clade B and clade C differentially induce DC-SIGN expression (Fig. 3). Furthermore, our results demonstrate that both clades induced oxidative damage and antioxidant enzyme activity; however, clade B Tat is significantly stronger than clade C Tat in inhibiting GSS, GPx1, SOD1 and catalase expression (Fig. 4). These results indicate HIV subtypes may affect redox-induced mRNA expression levels by different mechanisms in IDC.

Furthermore, the GSH/GSSG ratio plays a major role in maintaining cellular redox homeostasis. In IDC treated with clade B Tat, the GSH/GSSG ratio was markedly decreased compared with cells treated with clade C Tat (Fig. 5). Previous reports have indicated that HIV infection and HIV Tat protein down-regulate glutathione synthesis as evidenced by γ-GCS [51, 52], decreased GPx mRNA [32, 53], Mn SOD [44, 54] and catalase levels [55]. Our results demonstrated a significant increase in clade B-induced oxidative stress, which inhibited transcription of redox genes and proteins modification (Fig. 6). Interestingly, there was no significant change in redox gene expression in response to clade C Tat. Recent studies have shown that clade B Tat induces continuous synthesis and secretion of cytokine, chemokine and neurotoxic factors, which may be involved in the observed effects [56, 57]. However, the structural and functional aspects of clade C Tat sequence at alterations in the dicysteine motif at position C30C31 in the clade C Tat protein likely decreases chemotactic function compared with clade B Tat [22]. Another study demonstrated that clade B Tat binds CCR2, whereas clade C Tat does not [58]. Campbell et al. demonstrated that when clade B was bound, it induced significantly more pro-inflammatory cytokines TNF alpha and CCL2 receptors in monocytes [59]. TNF-α is elevated during HIV progression and is negatively correlated with GSH and also serves as a second messenger in oxidative stress [25, 60]. Also, studies have demonstrated that depleted GSH in HIV positive individuals [61] play a major role in the apoptosis of $CD4^+$ T cells [62]. Furthermore, GSH levels in DC which maintains redox homeostasis and protecting DCs from oxidative stress are highly specialized in their ability to process and present exogenous antigen to CD4+ T helper cells and endogenous antigen to CD8+ T cells and accordingly influence the immune response by Th1 cytokine secretion [63–65]. The present observations indicate that redox gene expression modulates immune cells as well as the neuronal response during clade B infection.

Increases in the expression of various pro-inflammatory genes have been shown to be dependent on the cellular GSH level [66], and in turn, GSH levels are regulated by oxidative stress and redox balance and are known to promote neuropathogenesis [67]. These findings suggest that GSH plays a role in the development of HAD/HAND. Our recent studies

showed that HIV-1 clade B Tat induced significantly higher levels of neurotoxin kynurenine in astrocytes and dendritic cells than clade C Tat [46, 56]. Another study reported that oxidative stress induces quinolinic acid (68), which then inhibits antioxidant enzymes and redox expression (Figure 7). Therefore, it is possible that the secretion of immunopathogenic molecules during HIV infection leads to GSH dysregulation and ROS production. Further studies are needed to fully define the mechanism of clade B and C Tat-induced redox gene expression and enzyme activation. Overall, the data provide evidence that there is a connection between redox gene and protein expression enhancing the level of ROS production and inhibiting intracellular signaling mechanisms in clade B and clade C Tat-treated cells. The down regulation of redox gene expression by the Tat protein may lead to increased oxidative damage and ultimately cell death. The present study supports the idea that oxidative stress and redox-dependent gene expression are associated with immune dysfunction, particularly with the reduction of GSH [69].

In conclusion, infection with HIV-1 clade B and C or treatment with the respective clade-specific Tat proteins modulated the GSH/GSSG ratio and down-regulated GSS, GPx1, SOD1 and catalase in IDC and neuronal cells. Interestingly, we also demonstrated that clade B and C infection and Tat treatment differentially inhibited redox expression, leading to immune cellular dysfunction and perhaps potentiation of neuronal disease progression. To the best of our knowledge, this is the first report that HIV-1 clade B has a greater impact on oxidative stress than clade C. Taken together; these results suggest that clade-specific mechanisms in cellular oxidative stress might play a critical role in the immuno-neuropathogenesis of HAND.

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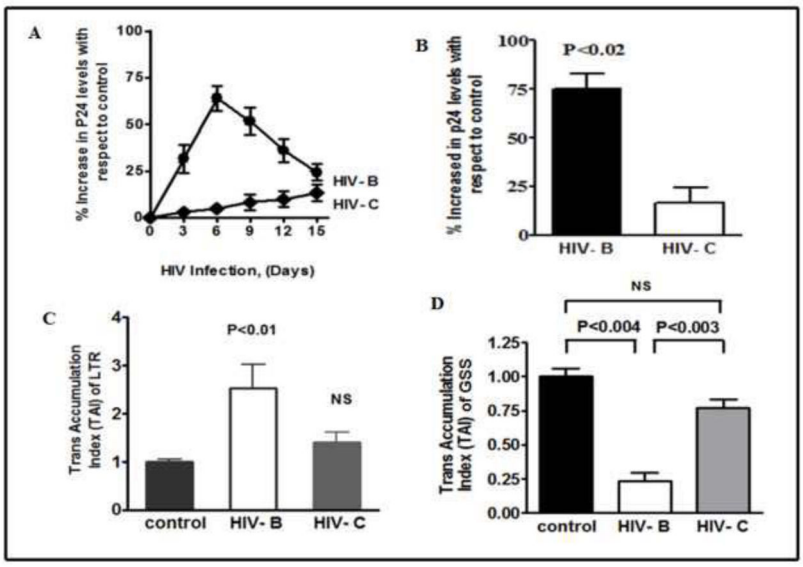

Fig. 1.

The effects of HIV-1 clade B and C viruses on IDC. IDC (1×10^6 cells/ml) were infected with HIV-1 clade B (Bal strain) or HIV-1 clade C (CN54 strain) at 20ng/10^6 cells (A) or $TCID_{50}$ (B) for 18 hours. The supernatants were collected at different time points (A) and 6 hr (B) used to estimate the expression of p24 antigen by ELISA. Total RNA was extracted from the infected IDC and then reverse transcribed and subjected to quantitative real time PCR using gene-specific primers to measure the expression of LTR (C), GSS (D) and the housekeeping gene β-actin. The data are expressed as the mean ± SD of the transcript accumulation index (TAI) values of three independent experiments.

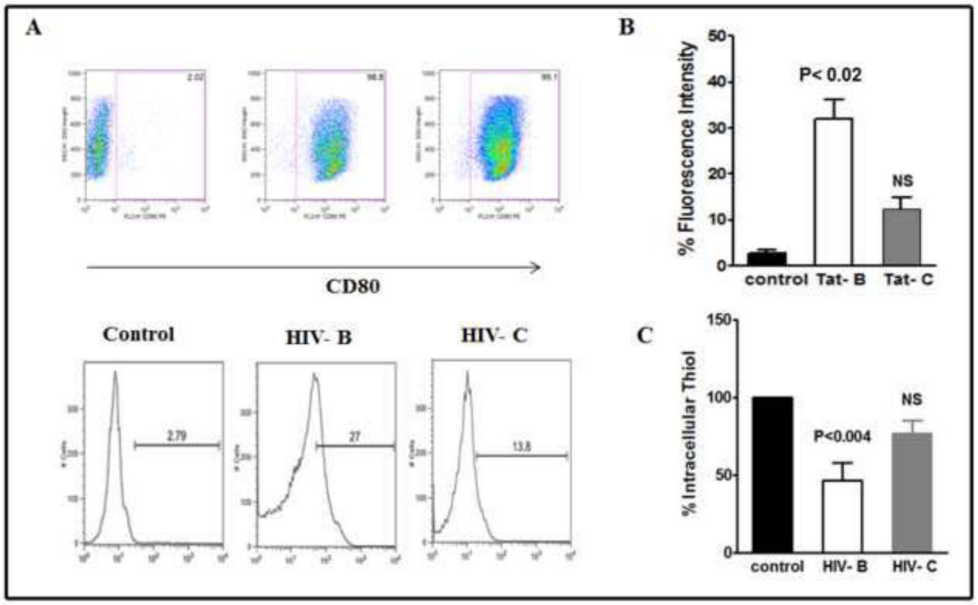

Fig. 2.

The effects of HIV clade B and C viruses on ROS production and intracellular thiol levels. IDC (1×10^6/ml) were seeded in six-well plates and infected with either HIV-1 clade B (Bal) or C (CN 54) (20 ng/ml) for 18 hours. Next, the cells were washed and maintain for 6 days. The IDC positive cells were stained for CD80 and ROS production by DCFH-DA (A), H_2O_2 by Amplex Red (B) and intracellular thiol content by mBBr (C) and analyzed by flow cytometry. The data are expressed as the mean ± SD of three independent experiments.

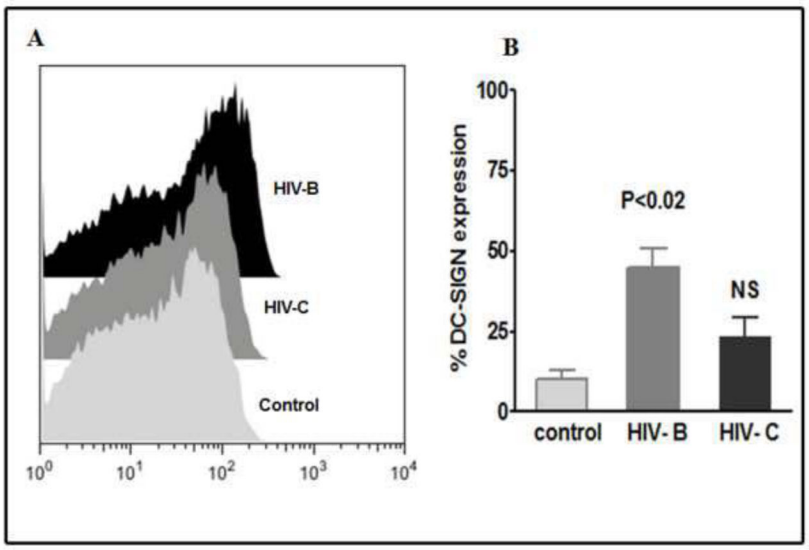

Fig. 3.

The effects of HIV clade B and C viruses on DC-SIGN expression in IDC. IDC (5×10^5 cells/ml) were cultured and infected with either HIV-1 clade B (Bal) or C (CN 54) (20 ng/ml) for 18 hours. The cells were then washed to remove unbound virus and cultured for 6 days. At the end of 6^{th} day, the cells were stained for DC-SIGN, and expression was analyzed by flow cytometry. The data are expressed as the mean ± SD of three independent experiments.

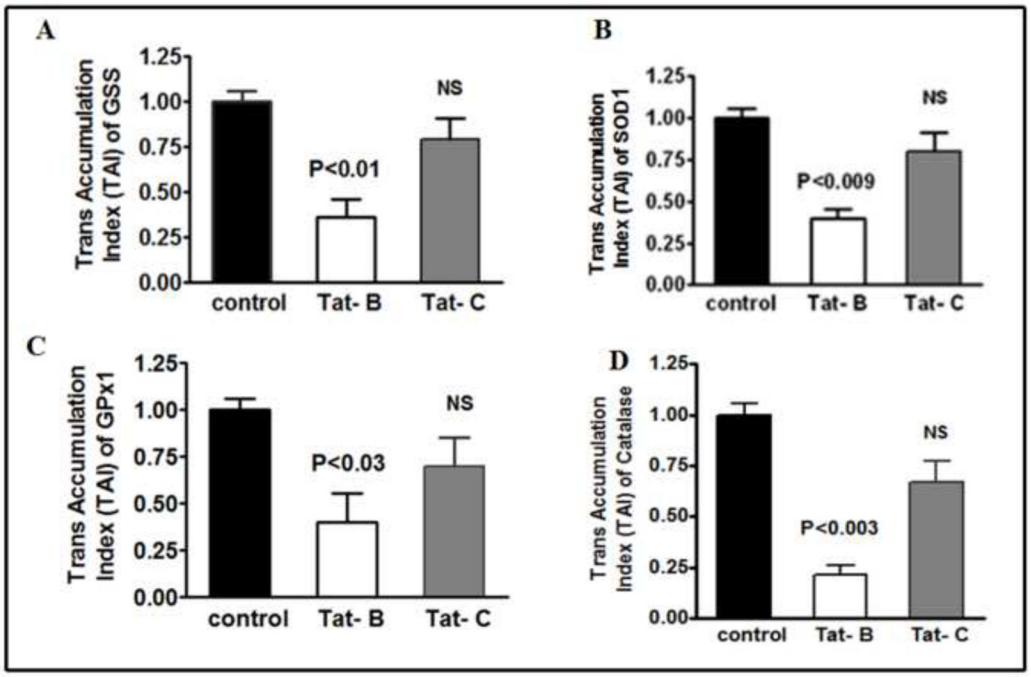

Fig. 4.

Differential effect of redox gene expression by HIV-1 clade B and C Tat protein. IDC (1 $\times 10^6$/ml) were treated with either HIV-1 clade B or clade C Tat protein (50 ng/ml) for 24 h. RNA was extracted and reverse transcribed followed by qRT- PCR for GSS (A), GPx1 (B), SOD1 (C), catalase (D) and the housekeeping gene β-actin. The data are expressed as mean ± SD of the transcript accumulation index (TAI) values of three independent experiments.

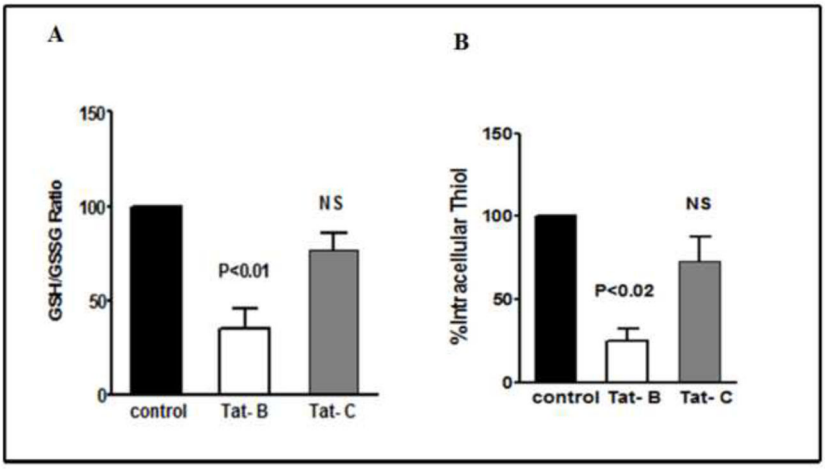

Fig. 5.

The effects of HIV-1 clade B and C Tat proteins on the GSH/GSSG ratio and intracellular thiol content. IDC (1×10^6 cells/ml) were seeded in six well plates and treated with either HIV-1 clade B or clade C Tat protein (50 ng/ml) for 24 h. The GSH/GSSG ratio was estimated spectrophotometrically (A), and the intracellular expression of thiol was determined by the percent of mBBr-positive cells (B). The data are expressed as the mean ± SD of three independent experiments.

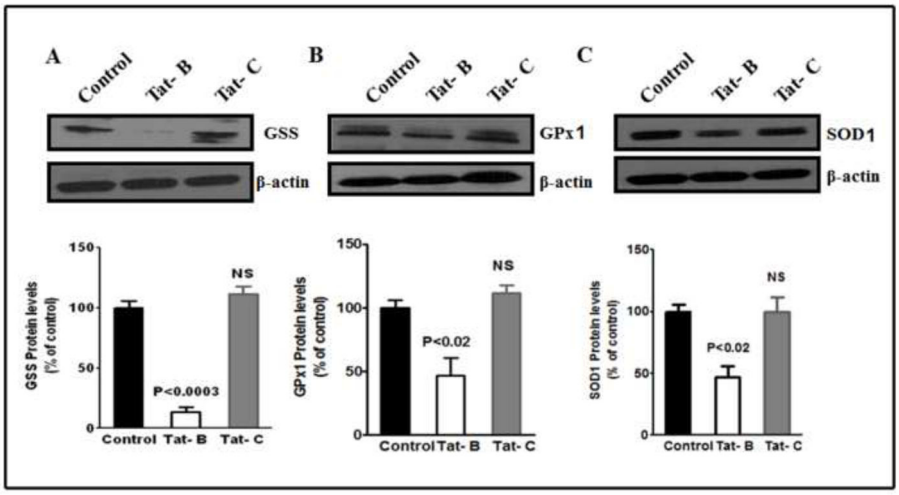

Fig. 6.

Differential effect of redox proteins by HIV-1 clade B and C Tat protein. IDC were treated with either HIV-1 clade B or clade C Tat protein (50 ng/ml) for 24 h and analyzed by Western blot to detect GSS (A), GPx1 (B) and SOD1 (C). The data are expressed as the mean ± SD of three independent experiments.

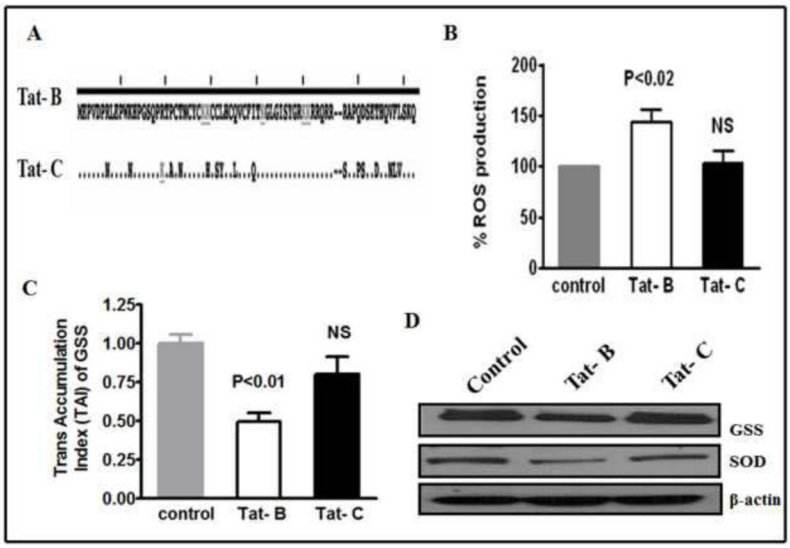

Fig. 7.

HIV clade B and clade C Tat sequence alignment and effect on SK-N-MC neuronal cells. Differences in the primary structure of clade B and clade C Tat sequence (A). SK-N-MC cells were cultured in six well plates and separately treated with either HIV-1 clade B Tat or clade C Tat (50 ng/ml) for 24 h. ROS production was analyzed by flow cytometry (B), RNA was extracted, reverse transcribed and then subjected to quantitative real time PCR using GSS-specific primers to measure GSS gene expression (C), and the cell lysates were resolved by western blot using GSS antibody (D). The data are expressed as the mean ± SD of three independent experiments.