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Regulation of topoisomerase II α and β in HIV-1 infected and uninfected neuroblastoma and astrocytoma cells: Involvement of distinct nordihydroguaretic acid sensitive inflammatory pathways ☆

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Abstract

The activity of Topoisomerase II α and β isoforms is tightly regulated during different phases of cell cycle. In the present study, the action of anti-inflammatory agents, nordihydroguaretic acid (NDGA) is analyzed in HIV-1 infected $CXCR4^+$, $CCR5^+$ and $CD4^-$ SK-N-SH neuroblastoma, $CXCR4^+$, $CCR5^+$ and $CD4^-$ 1321N1 astrocytoma and $CXCR4^+$, $CCR5^{+/-}$ and $CD4^-$ GO-G-CCM glioblastoma cell lines. In SK-N-SH and 1321N1 the expression of Topoisomerase II α is concomitant with that of LOX-5 and is highly sensitive to NDGA, while the Topoisomerase II β is expressed along with TNFα and exhibits low sensitivity to NDGA, suggesting distinct pathways of regulation for the two isoforms. HIV-1 infection in these cells enhanced the expression of Topo II α and β. Further, the regulation of Topo II β and TNFα in infected and uninfected SK cells is distinctly different. HIV-1 gp120 derived peptides could block HIV-1 mediated inflammation and Topoisomerase II α and β expression, suggesting the viral mediated response. A combination of NDGA, gp-120 derived peptides and AZT has completely blocked the viral replication, suggesting the enhancement of potency of AZT under the suppression of inflammatory response. In contrast, the expression of Topo II α and β was stimulated by NDGA in GO-G-CCM cells showing distinct regulatory pathway in these cells that was resistant to HIV-1 infection. This suggests the requirement of inflammatory response for productive viral infection. In summary, an induction of co-receptor mediated inflammatory response can distinctly enhance regulated expression of the cellular Topo II α and β and promote productive infection in neurons and astrocytes.
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Keywords: Neurons; Astroytes; Inflammation; Actin cleavage; Topoisomerase II; TNFα; gp120 peptides; NDGA; HIV-1

Neurological disorders are the first manifestation of symptomatic HIV-1[1] infection in 10–20% of population, while about 60% of seropositive persons with advanced HIV disease will have clinically detectable neurological dysfunction [1–3]. The incidence of sub clinical neurological disease is even higher along with peripheral neuropathy and HIV associated cognitive dysfunction leading to HIV associated dementia [4,5] in early infection. HIV crosses the blood brain barrier and enters the neuronal system with concomitant internal systemic infection [6]. HIV virus has been cultured from brain, nerve and cerebrospinal fluid (CSF) from patients during HIV infection [7,8]. 50% of patients with AIDS eventually show neurological complications directly attributable to the infection of the brain by the HIV-1. While HIV-1 viral turnover in circulation is primarily due to infection of $CD4^+$ cells, specifically T-lymphocytes and monocytes/macrophages, however, experimental evidence suggests that expression of the CD4 molecule is not the sole factor determining viral entry

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[1] *Abbreviations used:* HIV, human immunodeficiency virus; LOX-5, lipoxygense-5; TNFα, tumor necrosis factor α; IFNγ, interferon γ; HAD, HIV-associated dementia; NDGA, nordihydroguaiaretic acid; AZT, 3′-azido-3′-deoxythymidine; Topo II, topoisomerase II.

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[9]. Additionally, CD4 independent HIV-1 infection has been demonstrated in-vitro in several human cell lines and in primary human cell cultures [10]. These observations indicate that other cell surface molecules function as co-receptors in the presence of CD4 molecule or as alternative receptor in the absence of CD4. Recent findings *in vivo* indicated that $CD4^{-}$ cells in the brain, including astrocytes, endothelial cells and neurons harbor HIV-1 infection [11]. But some neurological cell types are sensitive to virus, while others are resistant. Several theories have been proposed regarding HIV-1 mediated neuropathogenesis, such as the aberrant cytokine production by HIV infected microglial and glial cells, neurotoxicity of gene products of virus (gp120 and tat) and alteration of function of astrocytes by cytokines and toxins produced by HIV gene products [12,13].

The cytokines TNFα and IFNγ have been implicated in the development and progression of multiple sclerosis (MS) and AIDS associated dementia complex. TNFα is a 17 KDa peptide produced by a wide range of cells [14]. TNFα plays an important role in (1) inflammation, (2) the modulation immunoresponse by affecting the expression of class I and class II MHC molecules and adhesion molecules, (3) stimulation of cytokines such as IL-1, IL-6, IL-8 and IFNγ [15]. TNFα participates in the inflammatory reaction within the CNS. TNFα positive macrophages and astrocytes have been identified in the brain of MS patients, particularly in the plaque region [16]. TNFα induction of IL-6 expression in astrocytes occurs by protein kinase C dependent pathway. Inflammatory markers shown to be enhanced in HIV-1 associated dementia, HIV-1 gp120 is shown to be involved in such response in neuroblastoma cells [17].

Topoisomerase II α (Topo II α) and Topoisomerase II β (Topo II β) are 170 and 180 KDa proteins; they promote the replication of viral DNA and chromosomal DNA [18]. Topo II α, is highly regulated during cell division, Topo II β is associated with non-proliferating function. The objective of the present investigation is to examine if systemic inflammation can regulate Topo II α and β in neuronal and astrocytes. The regulation of Topo II α and β were analyzed based on the sensitivity of their expression to NDGA. The results showed that inflammatory response in neurons and astrocytes regulate the expression of Topo II α and β in different pathways. The systemic inflammation in neurons and astrocytes was also induced as a stress response related to the HIV-1 infection and the results showed the regulation of induction of inflammatory response for productive HIV-1 replication mediated by cellular receptor CCR5 in neurons, astrocytes and glial cells.

Materials and methods

The following reagents were obtained from AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. The reagent contributor name is given in parenthesis. SupT1 cell line (Dr. J. Hoxie). HIV-1 virus subtype C, HIV-$1_{93IN101}$ (Dr. R. Bollinger), pNL4-3 (Dr. Malcolm Martin), SK-N-SH (Neuroblastoma), GO-G -CCM (Glioblastoma) and 1321N1 (astrocytoma) cell line were obtained from NCCS, Pune, INDIA.

V3-peptide (TRPNYNKRKRIHIGPGRAFYTTKNIIGTIRQAH-NH2., (5), MN-peptide (306-327 YNKRKRIHIQRGPGRAFYTTKNII (C),. [19,20] from NIH USA.

Mouse anti human Topoisomerase II α and β were from BD biosciences. Monoclonal anti-human CD4, CXCR4 and CCR5 from NIH USA. Monoclonal anti-human TNFα, Rabbit anti-human COX-2 and LOX-5 from US biological. The secondary antibodies were from UPSTATE USA. NDGA and AZT from Sigma–Aldrich. Each experiments was repeated three times. ELISA assays were carried out in triplicates. Data was plotted as an average of triplicates with standard deviation.

Cell culture

SK-N-SH neuroblastoma cell line was maintained in EMEM with 0.1 mM non-essential amino acids, 0.01 mM sodium pyruvate and 10% fetal bovine serum, GO-C-CCM and 1321N1 cell line were maintained in DMEM with 10% FBS. SupT1 cell line were maintained in RPMI with 10% FBS.

FACS analysis

The FACS analysis was done according to Bruce et al. (1990) [21] Briefly to prepare cells for fluorescence-activated cell sorter (FACS) analysis, adherent cells were removed from plastic tissue culture flasks by trypsinization for 8 min at 37 °C. Fetal calf serum was added to 25% to block trypsin activity. The cells were then filtered through nylon mesh to remove clumps, counted, and distributed to a 96-well tray (5×10^5 cells per well) in 50 µl of RPMI 1640 medium containing 0.01 M NaN_3, washed twice with RPMI-azide, and then incubated with mouse anti-human monoclonal antibodies (1:50 dilution) CD4, CXCR4 and CCR5 in 50 µl of RPMI-azide for 1 h at 4 °C. After two washes with RPMI-azide, cells were incubated in 150 µl of 1:180 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin in RPMI-azide for 1 h at 4 °C. after two washes with RPMI-azide, the cells were suspended in 1 ml of PBS containing 1% formaldehyde. The stained and fixed cells were analyzed on a FACSTAR cell sorter (Becton Dickinson, Mountain View, Calif.) with an argon ion laser emitting 488-nm light at 200 mW.

Infection assay

5×10^6 cells were seeded into the 60 mm dishes one day before the experiment, the cells were challenged with HIV-1 (20 ng/ml) in a medium containing 2% serum, and after 2 h post-infection, serum was increased to 10%. The infection was stopped at 4 and 24 h the time at which Topo II α and β and TNFα show significant expression.

For the p24 quantification the medium is aspirated out completely 48 h post-infection and cells were washed twice with the medium and supplemented with complete medium containing 10% FBS. HIV-1 was quantified in terms of p24 after 96 hours of post-infection.

HIV-1 infection in presence of NDGA and peptides

One day before the experiment, 5×10^6 cells were cultured in the 60 mm dishes. Drug and peptides were added to the cells at indicated concentrations and incubated for 10 min. These cells were challenged with HIV-1 as explained in infection assay.

Proviral DNA isolation

Cells (0.5×10^6) were challenged with HIV-$1_{93IN101}$ (200pg of p24 viral core protein) in the presence of peptides and NDGA at 5% CO_2 and 37 °C. The cells were harvested at 5 h p.i and washed with phosphate-buffered

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