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Human Immunodeficiency Virus-1 decreases the levels of neurotrophins in human lymphocytes

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Summary

Neurotrophins control cell survival. Therefore, we examined whether HIV-1 reduces neurotrophin levels. Serum of HIV-positive individuals exhibited lower concentrations of brain-derived neurotrophic factor (BDNF), but not of other neurotrophins, than HIV negative subjects. In addition, R5 and X4 strains of HIV-1 decreased BDNF expression in T cells. Our results support the hypothesis that reduced levels of BDNF may be a risk factor for T cell apoptosis and for neurological complications associated with HIV-1 infection.

Keywords

BDNF; NGF; NT-3; HIV-1; drug abuse; Women's Interagency HIV Study

Neurotrophins [1, 2] are produced by immune organs and immunocompetent cells including T cells [3] and macrophages [4], and are believed to play a role in various functions of the immune system including lymphocyte proliferation [5, 6]. Little is known about the effect of HIV-1 on neurotrophin levels. Loss of neurotrophin expression may impair the immune system and promote AIDS. In this work, we investigated whether HIV-1 reduces serum concentration of the neurotrophins and sought to establish a correlation between HIV infection and neurotrophin expression in T cells.

Serum levels of BDNF were measured by an Enzyme Linked Immunosorbent Assay (ELISA) in human samples collected between 1994–2007 at the Washington DC site of the Women's Interagency HIV Study [7, 8]. Because ~50% of these subjects were polydrug abusers, mainly cocaine, methamphetamine and heroin, a two-way ANOVA was used to examine a potential interaction between HIV-1 and drug use and to examine each factor independently. HIV-positive subjects exhibited significantly lower levels of BDNF compared to HIV negative controls (Fig. 1A). Drug use significantly affected BDNF levels

such that the amount of BDNF in the serum of HIV-positive drug users were higher than in HIV-positive non-drug users (Fig. 1A), suggesting that polydrug use may affect serum BDNF levels in HIV-1 positive subjects. There was no interaction between drug use and serostatus on BDNF levels ($p>0.33$).

Drugs of abuse [9] or HIV-1 may influence the expression of other neurotrophins. To test this hypothesis we measured nerve growth factor (NGF) and neurotrophin-3 (NT-3) levels in the same samples. The two-way ANOVAs analyzing associations of HIV status and drug use on NGF ($p=0.516$) and NT-3 ($p=0.382$) were not statistically significant, and no evidence of interaction between HIV and drug use was observed for either outcome. Although we found a tendency toward lower average NGF levels in the serum of HIV positive subjects compared to controls, the effect was not significant ($p=0.89$), nor did polydrug use affect NGF levels (data not shown). Results for NT-3 levels were similarly not statistically significant (data not shown).

The reduction of BDNF observed in HIV-1 positive subjects could be due to single nucleotide polymorphisms (SNPs) that alter intracellular packaging and secretion of BDNF [10]. rs6265 is a polymorphism in the BDNF gene that produces an amino acid substitution valine to methionine in codon 66 (Val66Met); rs56164415 is located in the fifth of the seven non-coding exons of the BDNF gene [11] and appears to be moderately associated with substance abuse [12]. Therefore, these SNPs, either alone or in combination, might lead to a reduction in serum BDNF levels. To test this hypothesis we examined the frequency of these polymorphisms in the same cohort, using DNA from the same sample of subjects. There was no significant difference in frequency of alleles in HIV subjects as compared to HIV negative controls (rs6265, $p=0.83$, rs56164415, $p=0.72$). Therefore, mutation of the BDNF gene does not appear to account for difference in the levels of BDNF in these subjects.

Contributing factors that may account for the decrease in serum BDNF in HIV-positive subjects are not easily defined. BDNF and other neurotrophins are produced by immune organs and immunocompetent cells [13] as well as platelets [14]. Thus, a decrease in the number of platelets may explain the lower levels of BDNF in HIV-1 positive subjects. To determine whether BDNF from platelets constitutes a significant fraction of serum BDNF, we examined which blood cell type expresses BDNF. We found that platelets and T cells exhibited comparable levels of BDNF expression (Fig. 1B). Thus, platelets account for only for a fraction of serum BDNF. Nevertheless, to more directly examine the effect of HIV-1 on BDNF, we examined the ability of HIV-1 to decrease BDNF expression in T cells. T lymphocytes were prepared from healthy donors and were infected with X4 (IIIB) or R5 (BaL) HIVs. BDNF mRNA levels were then quantified 24 hr after the infection. We observed a ~50% decrease in BDNF mRNA levels by both HIV-1 strains (Fig. 1C) further suggesting that HIV-1 is capable of reducing the expression of this neurotrophin in T cells.

Our main finding is that the serum of HIV-positive women is characterized by reduced levels of BDNF but not of NGF or NT-3, irrespective of drug use status, suggesting that HIV-1 influences the expression of selected neurotrophins. This was confirmed by direct evidence that both R5 and X4 HIV-1 strains down-regulate BDNF mRNA levels in T cells. These results may contribute new insights into our understanding of the immune dysregulation of AIDS. In fact, given the well-known antiapoptotic effect of the neurotrophins for T cells [6, 13], we may speculate that a decrease in BDNF could be among the mechanisms employed by HIV-1 to induce apoptosis of T cells. On the other hand, experimental evidence has shown an inverse correlation between levels of BDNF and CXCR4 [15] and CCR5 [16] expression. These co-receptors are crucial for HIV-1 infection [17]. Therefore, reduced levels of BDNF may be a risk factor for increasing HIV infection.

HIV-1 also causes axonal injury, neuronal loss and dementia [18]. BDNF is critical for neuronal survival [19]. Blood neurotrophin levels have been used to investigate the role of the neurotrophins in the pathogenesis of various neurodegenerative diseases. In fact, recent data have shown a relationship between BDNF in blood and Alzheimer's disease [20] and age-related cognitive impairment [21]. Therefore, serum BDNF could be a predictor of risk for the development of neurological signs in HIV-positive individuals. Our findings of an association between HIV infection and serum BDNF levels, and of lowered BDNF mRNA levels in infected T cells, provide initial evidence in support of this hypothesis and suggest this neurotrophin as a possible biomarker for HIV dementia. Additional studies are needed to validate our results and extend them to both genders, as we examined a relatively small cohort of women subjects. Also a link between BDNF and cognitive performance needs to be established.

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Figure 1. HIV-1 and BDNF levels

A. The amount of BDNF was determined in the serum of HIV positive (n=109) and negative (n=54) individuals, non drug or drug abusers by ELISA according to the manufacturer's instructions (Promega Corp.). The study was approved by Georgetown University Institutional Review Board. HIV and drug use status did not interact [F(1, 155) = 0.076, p=0.783]. However, main effects of HIV status [F(1, 155) = 5.203, p=0.024] and drug use [F(1, 155) = 7.27, p=0.008] were observed. **B.** BDNF mRNA levels were determined by real-time quantitative polymerase chain reaction (RT-PCR) in the indicated cells obtained from peripheral blood mononuclear cells taken from healthy volunteers. Primers for BDNF were 5'-CATTGGCTGACACTTTCGA-3' and 5'-ACTGAGCATCACCTGGAC-3' (forward, reverse). Hypoxanthine phosphoribosyltransferase 1 was used as a housekeeping gene. Data are the mean $\pm$ SEM of three independent samples. **C.** Human T cells (0.5×10^6 cells/well) prepared as previously described [22] were infected for 2 hr with ~ 100 TCID₅₀ of HIV_{BaL} or HIV_{IIIb}. Infection was monitored by p24 ELISA (PerkinElmer Life Sciences, Inc.). BDNF mRNA levels were determined 24 hr after the infection by RT-PCR. Reverse-transcriptase negative controls were used to exclude genomic DNA contamination. Data, expressed as % of untreated cells, represent the mean of two independent preparations.