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Proteomic analyses of methamphetamine (METH)-induced differential protein expression by immature dendritic cells (IDC)☆

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Abstract

In the US, the increase in methamphetamine (METH) use has been associated with increased human immunodeficiency virus (HIV-1) infection. Dendritic cells (DC) are the first line of defense against HIV-1. DC play a critical role in harboring HIV-1 and facilitate the infection of neighboring T cells. However, the role of METH on HIV-1 infectivity and the expression of the proteome of immature dendritic cells (IDC) has not been elucidated. We hypothesize that METH modulates the expression of a number of proteins by IDC that foster the immunopathogenesis of HIV-1 infection. We utilized LTR amplification, p24 antigen assay and the proteomic method of difference gel electrophoresis (DIGE) combined with protein identification through high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to analyze the effects of METH on HIV-1 infectivity (HIV-1 IIIB; CXCR4-tropic, X4 strain) and the proteomic profile of IDC. Our results demonstrate that METH potentiates HIV-1 replication in IDC. Furthermore, METH significantly differentially regulates the expression of several proteins including CXCR3, protein disulfide isomerase, procathepsin B, peroxiredoxin and galectin-1. Identification of unique, METH-induced proteins may help to develop novel markers for diagnostic, preventive and therapeutic targeting in METH using subjects.

Keywords: Methamphetamine; Immature dendritic cell (IDC); Difference Gel Electrophoresis (DIGE); HPLC-MS/MS

1. Introduction

Methamphetamine (METH) use is associated with a high risk of contracting human immunodeficiency virus (HIV-1) infection as a result of sharing contaminated needles and increased, risky, sexual behavior [1–7]. In 2005, an estimated 10.4 million Americans aged 12 and above tried METH at least once in their lifetime [2]. In the US METH used as a recreational drug has surpassed cocaine as of July 2005 [8]. Little is known about METH's effects on the host's immune response and the immunopathogenesis of HIV-1 infections.

Dendritic cells (DC) are antigen presenting cells that are the first line of defense against all pathogens including HIV-1 [9–15]. Immature DC (IDC) specialize in capturing and processing antigens. Interaction of IDC with an antigen results in cellular activation or maturation and migration to regional lymphoid tissues where the processed antigens are presented to naive CD4+ T cells, subsequently enabling T cell activation [13,15]. Although the ability of DC to present antigen and stimulate naive T cells has been shown in vitro, a better understanding of the immunoregulatory activities of DC on the immune system and their role in HIV-1 infection remains to be determined. A number of proteins induced by drugs of abuse have been implicated in susceptibility to HIV-1 infection and progression of the disease [16,17]. There exists substantial literature on the psychosocial aspects of METH abuse and

Abbreviations: HSPA8, Hsp70, protein 8 isoform 1; IDC, immature dendritic cells; MDC, mature dendritic cells; PDI, protein disulfide isomerase; HSPA5, dnaK-type molecular chaperone

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susceptibility to infection with HIV-1. However it is further suggested that exposure to METH also may have a biological effect on increased susceptibility to and progression of HIV-1 infections [4–7,18]. These studies support our premise that METH has a direct effect on the ability of HIV-1 to infect its primary target, IDC. Furthermore we investigated the pathophysiologic mechanisms underlying this effect by examining METH-induced differences in protein profiling of IDC cultures using LTR amplification, p24 antigen assay and the proteomic method of difference gel electrophoresis (DIGE). The identification of unique, METH-responsive proteins by proteomic analyses may distinguish novel biomarkers for the prevention, diagnosis and treatment of HIV-1 infections of METH-using patients.

2. Methods

2.1. Isolation and generation of DC

Blood donors were apprised of this study and consents were obtained consistent with the policies of the appropriate local institutions and the National Institutes of Health. Peripheral blood samples from healthy, HIV-1 negative individuals were drawn into a syringe containing heparin (20 units/ml). DC were prepared as described [19–22]. Briefly, human peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ). $CD14^+$ monocytes were separated from PBMCs using plastic adherence; the purity of monocytes was >90%, verified using flow cytometry for CD14 (data not shown). To prepare immature DC (IDC), monocytes were cultured in RPMI 1640 medium with 1% human, AB serum, 500 U/ml of recombinant human interleukin-4 (IL-4; R&D Systems, Minneapolis, MN), and 1000 U/ml of recombinant human granulocyte-macrophage colony-stimulating factor (GMCSF, Immunex) for 48 h. Mature DC (MDC) were prepared by culturing IDC for an additional 2 days in the presence of interleukin-1 β (IL-1β, 10 ng/ml, R&D Systems, Minneapolis, MN), interleukin-6 (IL-6, 1000 U/ml, R&D Systems), tumor necrosis factor (TNF, 10 ng/ml, R&D Systems) and prostaglandin E_2 (PGE_2, 1 μg/ml, Sigma-Aldrich, St. Louis, Mo.).

Both IDC and MDC were washed in FACS buffer (e-Bioscience, San Diego, CA), incubated with nonspecific human IgG (20 μg/ml) for 10 min at 4 °C to block Fc receptors, stained with specific antibodies for DC surface markers and analyzed by flow cytometry. Both MDC and IDC express CD80, CD86, and CD83 at different levels (Fig. 1). However, MDC predominantly express CD83 as described [23]. IDC were used for all experiments since earlier studies have shown that IDC are more susceptible to HIV-1 infection than MDC [24,25]. IDC were >98% viable by trypan blue exclusion criteria.

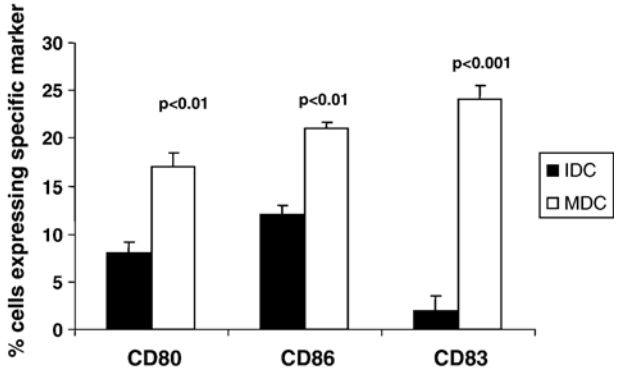

Fig. 1. Constitutive expression of biomarkers on mature (MDC) and immature (IDC) dendritic cells as measured by FACS analysis. Statistical significance was determined by Student's *t*-test ($n=3$ independent experiments).

2.2. Drug treatment

IDC were treated with METH (Sigma-Aldrich) at 50, 100 and 250 μM for 24 h. The concentrations of METH used were based on previous dose response studies (1 to 250 μM) that produced a maximum biological response without causing toxicity to the target cells and also were based on published in vitro studies [26,27]. These concentrations are similar to levels found in blood, urine or tissue samples of METH users that range from ≤2 μM to 600 μM [28–31]. METH was dissolved in sterile distilled water and was subsequently diluted in media to the required concentrations. For all experiments, cells treated with vehicle alone (media) were used as the untreated control.

2.3. Infection of human IDC with HIV-1

IDC (1×10^5 cells/ml) were treated with METH (100 μM) for 24 h and infected with native HIV-1 IIIB (CXCR4-tropic, X4 strain; NIH AIDS Research and Reference Reagent Program, Cat #398) overnight at a concentration of $10^{3.0}$ $TCID_{50}$/ml cells, and washed 3 times with Hank's balanced salt solution (Invitrogen, Grand Island, NY) before being returned to culture. A post infection period of 48 h was used to amplify the LTR-R/U5 region that represents early stages of reverse transcription of HIV-1 [32]. In separate experiments, IDC were treated with METH for 24 h, infected with HIV-1 IIIB overnight and washed and cultured for 15 days. The culture supernates were assayed for p24 antigen using a p24 ELISA kit (ZeptoMetrix Corporation, Buffalo, NY) on day 15.

2.4. RNA extraction and real time, quantitative PCR (Q-PCR)

IDC treated with and without METH (100 μM, 24 h) were washed with 1X PBS (Invitrogen) and cytoplasmic RNA was extracted using an acid guanidinium–thiocyanate–phenol–chloroform method [33]. The final RNA pellet was dried and resuspended in diethyl pyrocarbonate (DEPC) water and the concentration of RNA was determined using a spectrophotometer at 260 nm. Any DNA contamination in the RNA preparation was removed by treating the RNA with DNAse (1 IU/μg of RNA, Promega, Madison WI) for 2 h at 37 °C, followed by proteinase K digestion at 37 °C for 15 min and subsequent extraction with phenol/chloroform and NH_4OAc/ETOH precipitation. The isolated RNA was stored at −70 °C until used. DNA contamination of the RNA preparation was checked by including a control in which reverse transcriptase enzyme was not added to the PCR amplification procedure. Gene expression for HIV-1 LTR RU/5, filamin 1, talin 1, CXCR3, HSPA8, PDI, enolase, thioredoxin, nuclear ribonucleoprotein A2/B1, aldolase A, cathepsin X, inorganic pyrophosphatase, cathepsin B, galectin-1, calreticulin, pyruvate dehydrogenase, pyruvate kinase, coronin actin binding protein 1A, tyrosine 3/tryptophan 5-monooxygenase activation protein, zeta polypeptide, HSPA5, peroxiredoxin, 18 s (Ambion, Austin, TX) and β-actin were quantitated using real time Q-PCR (Table 1). Relative abundance of each mRNA species was assessed using the SYBR green master mix from Stratagene (La Jolla, CA) to perform Q-PCR using the ABI Prism 5700 instrument that detects and plots the increase in fluorescence versus PCR cycle number to produce a continuous measure of PCR amplification. To provide precise quantification of initial target in each PCR reaction, the amplification plot is examined at a point during the early log phase of product accumulation. This is accomplished by assigning a fluorescence threshold above background and determining the time point at which each sample's amplification plot reaches the threshold (defined as the threshold cycle number or C_T). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube [34]. Relative mRNA species expression was quantitated and expressed as transcript accumulation index ($TAI=2^{-(\Delta\Delta CT)}$), calculated using the comparative C_T method [35]. All data were controlled for quantity of RNA input by performing measurements on an endogenous reference gene, β-actin. In addition, results on RNA from treated samples were normalized to results obtained on RNA from the control, untreated sample.

2.5. Calculation of transcript accumulation index (TAI) or relative expression

For each sample, a difference in C_T values (ΔC_T) was calculated for each mRNA by taking the mean C_T of duplicate tubes and subtracting the mean C_T of