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HIV and symptoms of depression are independently associated with impaired glucocorticoid signaling

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ABSTRACT

Chronic inflammation caused by HIV infection may lead to deficient glucocorticoid (GC) signaling predisposing people living with HIV to depression and other psychiatric disorders linked to GC resistance. We hypothesized that comorbid HIV and depressive symptoms in women would synergistically associate with deficits in GC signaling. This cross-sectional study used samples obtained from the Women's Interagency HIV Study (WIHS). The Centers for Epidemiological Studies (CES-D) was used to define depression in four groups of women from the Women's Interagency HIV Study (WIHS): 1) HIV-negative, non-depressed (n = 37); 2) HIV-negative, depressed (n = 34); 3) HIV-positive, non-depressed (n = 38); and 4) HIV-positive, depressed (n = 38). To assess changes in GC signaling from peripheral blood mononuclear cells (PBMCs), we examined baseline and dexamethasone (Dex)-stimulated changes in the expression of the GC receptor (GR, gene: *Nr3c1)* and its negative regulator *Fkbp5* via quantitative RT-PCR. GR sensitivity was evaluated *in vitro* by assessing the Dex inhibition of lipopolysaccharide (LPS)-stimulated IL-6 and TNF-α levels. Depressive symptoms and HIV serostatus were independently associated with elevated baseline expression of *Fkbp5* and *Nr3c1*. Depressive symptoms, but not HIV status, was independently associated with reduced LPS-induced release of IL-6. Counter to predictions, there was no interactive association of depressive symptoms and HIV on any outcome. Comorbid depressive symptoms with HIV infection were associated with a gene expression and cytokine profile similar to that of healthy control women, a finding that may indicate further disruptions in disease adaptation.

1. Introduction

Advances in antiretroviral therapy (ART) have dramatically increased the life expectancy of people living with HIV (PLWH); however, PLWH still suffer from inflammation-related diseases such as cardiovascular disease, type II diabetes, cancer, and dementia at higher

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rates and at younger ages than uninfected individuals (Nemeth et al., 2015; Valdez et al., 2016). Although it has been established that persistent immune activation and inflammation contribute to non-AIDS pathologies in PLWH who are on suppressive antiretroviral therapy (ART) (Hunt, 2012), the factors contributing to sustained inflammation in otherwise healthy PLWH have not been identified.

Depression co-morbid with HIV infection has emerged as a predictor of HIV-related comorbidities (Kelso-Chichetto et al., 2018; Rivera-Rivera et al., 2014, 2016) and increased mortality (Ickovics et al., 2001). Although the precise mechanistic relationship between depression and HIV-related comorbidities has not been elucidated, the biological relationship between inflammation and depression may be a critical factor (Valdez et al., 2016). In seronegative individuals, a bi-directional relationship between depression and inflammation has been established such that inflammation increases the risk of depression (Miller et al., 2009) and psychosocial stress and negative mood increase inflammation (Glaser and Kiecolt-Glaser, 2005). Although not as well characterized in the context of HIV infection, depression and HIV-related comorbidities are more prevalent in women than in men (Kelso-Chichetto et al., 2018), and middle-aged women living with HIV (WLWH) are at increased risk for systemic immune activation/inflammation compared to men living with HIV (MWLH) (Raghavan et al., 2017).

A potential mechanism that may lead to pervasive inflammation and has been implicated in the pathophysiology of depression, is dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis (Bekhbat et al., 2017; Neigh and Nemeroff, 2006). Glucocorticoids (GC) such as cortisol (CORT), are main effectors of the HPA axis. Although previous studies have demonstrated dysfunction of the HPA axis in PLWH (Patterson et al., 2013), these studies have focused only on the ligand, CORT, and not the GC receptor (GR) specifically. Through direct action as a transcription factor and via transrepression of pro-inflammatory transcription factors, the GR suppresses inflammatory signaling. Conversely, reduced function of the GR can precipitate elevated inflammation (Bekhbat et al., 2017).

We sought to test the central hypothesis that depression and HIV infection interact to alter the function of the GR and precipitate an enhanced inflammatory response. Hyperactivity of the HPA axis observed in depression is commonly accompanied by decreased sensitivity of GRs as evidenced by reduced translocation of GR to the nucleus and reduced transcriptional activity (Pariante and Miller, 2001). Chronic inflammation caused by HIV infection may lead to deficient GC signaling, thus predisposing PLWH to psychiatric disorders linked to GC resistance, such as depression (Pace et al., 2007). We hypothesized that comorbid symptoms of depression in PLWH would synergistically associate with deficits in GC signaling, demonstrated to be a sensitive marker of GC resistance in depressed patients (Menke et al., 2012), both in the absence of GC stimulation and when stimulated with a synthetic GC, dexamethasone (Dex). In addition to direct assessment of GR, we also assessed a negative regulator of GR, FK506-binding protein 51 (FKBP5), and a postivie regulator, FK506-binding protein 52 (FKBP4) (Davies et al., 2002). Importantly, expression of FKBP5 and its genetic variations have been linked to a number of psychiatric conditions involving HPA axis dysfunction including depression (Menke et al., 2013), post-traumatic stress disorder (Klengel et al., 2013), and chronic stress (Hartmann et al., 2012). In addition to the established influence of FKBP5 on the GR, FKBP5 has been shown to play a role in HIV-associated cognitive deficits in PLWH (Soontornniyomkij et al., 2012; Tatro et al., 2009).

2. Methods

2.1. Participants

This cross-sectional study used PBMCs obtained from participants in the Women's Interagency HIV Study (WIHS). WIHS began in 1993, and is a comprehensive multisite prospective cohort study designed to investigate the progression of HIV disease in women. WIHS is funded to the National Institutes of Health. Study visits occur every six months and include detailed interviews, physical examinations, and laboratory testing. Participants were selected as members in one of four groups: 1) HIV-negative, no depressive symptoms, based on CES-D < 16) (n = 37, "HIV-/No-Dep"); 2) HIV-negative, depressive symptoms (n = 34, "HIV-/Dep"); 3) HIV-positive, no depressive symptoms (n = 38, "HIV +/No-Dep"); and 4) HIV-positive, depressive symptoms (n = 38, "HIV +/Dep"). All participants were younger than 45 years old and African-American. Samples were excluded if the contributing subject had an AIDS diagnosis or active AIDS-defining opportunistic conditions secondary to HIV infection, current use of hormone-mediated contraceptives, was pregnant or nursing, met DSM-IV criteria for current substance/alcohol abuse or dependence (drank ≥12 glasses of alcohol/week or non-marijuana drug use [cocaine, crack, heroine, methadone, intravenous drug use]), seroconverted from HIV-negative to HIV-positive during WIHS follow-up, or had used antipsychotic, antidepressant, or antipsychotic medication or mood stabilizers within the past 4 weeks. A score of 16 or higher on the Center for Epidemiologic Studies Depression Scale (CES-D) was used to indicate a clinically relevant depressive symptom burden. Due to the high prevalence of marijuana use among HIV-/No-Dep group (approximately 35%), the four study groups were frequency-matched on marijuana use to ensure equal distribution of this potential confounder. Groups were similar on HIV RNA status (detectable/not detectable), HCV status, age, race, and $CD4^+$ count.

2.2. Cell culture

PBMCs were cryopreserved in liquid nitrogen until plated. Cells were thawed in an RPMI medium supplemented with 5% FBS, 2 mM L-glutamine, 10/10 pen/strep, and plated in duplicate at a density of 10^6/mL per well of a 12-well cell culture plate (Corning Inc, Corning, NY). Following overnight incubation in a humidified atmosphere at 37 °C in 5% CO_2, the cells were stimulated with vehicle (baseline), dexamethasone (Dex, Sigma Aldrich, product D-4902), lipopolysaccharide (LPS, Sigma Aldrich, product L2880), or concurrent Dex and LPS. Dex was given at 0 h at a concentration of 10^{-8} M, and LPS was given at 6 h at a concentration of 100 ng/mL. Cells were harvested at 12 h, centrifuged at 1000rcf for 5 min at 4 °C, and following a wash with ice-cold PBS, the cell pellets were lysed for RNA extraction. Cell lysates and cell culture supernatant were stored at −80 °C until used for RNA extraction and ELISA.

2.3. Quantitative PCR

RNA was extracted from cell pellets using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNA integrity was assessed by a Nano-Drop 2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA) and RNA samples were reverse transcribed using the High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, CA, USA). To ensure uniform amounts of total cDNA across groups, cDNA was quantified via the PicoGreen Assay (Invitrogen, Carlsbad, CA), then standardized, so that all samples started quantitative RT-PCR with 0.1 μg cDNA. The human gene Rpl13a was determined to be the optimal endogenous control based on an inter-group variance of less than 10% across groups. Primers for Fkbp5 (forward: CTTGCTGCCTTTCTGAACCT, reverse: CCCTTGGCTGACTCA AACTC), Nr3c1 (forward: CGAGCATGAGACCAGATGTA, reverse: CGA CTGCTCTTTTGAAGAAA), Fkbp4 (forward: AAGCTGGAACAGAGCAC CAT, reverse: GCAGCAGAGAAGGCCTGTAG), Rpl13a (forward: ATGC TGCCTCACAAGACCA, reverse: TAGGCTTCAGACGCACGAC) were designed and purchased from Applied Biosystems (Foster City, CA). The universal two-step RT-PCR cycling conditions used on the 7900 H T Sequence Detection System (Applied Biosystems, Foster City, CA) were: