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Drug use is associated with anti-CD4 IgG-mediated CD4+ T cell death and poor CD4+ T cell recovery in viral-suppressive HIV-infected individuals under antiretroviral therapy

Wei Jiang^{1,2,*}, Zhenwu Luo¹, Lisa Martin², Zhuang Wan¹, Pingfu Fu³, Amanda Wagner⁴, Binhua Ling⁵, Sonya L. Heath⁶, Azizul Haque¹, and Aimee McRae-Clark^{4,7}

¹Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, USA, 29425

²Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, Charleston, USA, 29425

³Department of Population and Quantitative Health Science, Case Western Reserve University, Cleveland, OH 44106

⁴Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, USA, 29425

⁵Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA, 70112; Tulane National Primate Research Center, New Orleans, LA, 70433

⁶Division of Infectious Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA, 35294

⁷Ralph H. Johnson VA Medical Center, Charleston, USA 29403

Abstract

Background.—The role and mechanism of drug use or abuse in antiretroviral therapy (ART)-treated HIV disease are not completely known.

Methods.—To investigate the impact of drug use on HIV pathogenesis without confounding by HIV replication and ART adherence, we first analyzed the data from our clinical database in 103 HIV+ subjects with viral-suppressed ART treatment by a multiple regression test.

Results.—We found that HIV+ drug users had lower CD4+ T cell counts but higher CD8+ T cell counts compared to HIV+ non-drug users, and both drug use and nadir CD4+ T cell counts was independently associated with CD4+ T cell recovery after controlling for sex and age. Next, we enrolled individuals from four study groups, HIV-negative and HIV+ subjects without any substance use, HIV-negative and HIV+ subjects with current illicit drug use (either non-injection cocaine or cannabis). All HIV+ subjects were viral-suppressed with ART treatment (> 2 years). Notably, HIV+ drug users had increased plasma anti-CD4 IgG levels compared to the other three

*Corresponding author: Wei Jiang, 173 Ashley Ave. BSB208D, Charleston, SC, 29425; Phone number: 843-876-2457, jjianw@musc.edu.

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study groups which were inversely correlated with decreased CD4+ T cell counts only in drug use in HIV disease. There was a significant increase in CD4+ T cell recovery following ART in HIV+ non-drug users but not in HIV+ drug users. Anti-CD4 IgGs purified from plasma of HIV+ drug users induced CD4+ T cell death *in vitro* through antibody-dependent cytotoxicity (ADCC).

Conclusion.—These results suggest that drug use prevents immune reconstitution in HIV-infected individuals despite long-term ART treatment and viral suppression.

Keywords

HIV; drug use; antiretroviral therapy; anti-CD4 IgG; CD4+ T cell recovery; antibody-dependent cytotoxicity

Introduction

Antiretroviral therapy (ART) dramatically restores CD4+ T cells, improves immune function, suppresses HIV viral replication, delays disease progression, and increases survival in HIV disease [1-3]. However, a proportion of patients (up to 25%) cannot restore their peripheral CD4+ T cell counts under viral-suppressive ART [2, 4, 5]; as a consequence of low CD4+ T cell counts, these patients exhibit increased inflammation, morbidity and mortality [3, 6-10]. There are several mechanisms accounting for incomplete CD4+ T cell reconstitution, such as low nadir CD4+ T cell counts, dysfunction of thymic and lymphoid organs, elevated inflammation and immune activation driven by microbial translocation, and virus-mediated effects [11-20].

Drug use has shown to associate with increased inflammation [21, 22]. HIV infected drug users experience neurocognitive dysfunction mediated by increased systemic and local inflammation, as well as accelerated HIV disease progression [23-27]. Morphine, cocaine, methamphetamine, heroin, and amphetamine are the most frequently abused illicit drugs; and illicit drug use is associated with increased rate of HIV infection, comorbidities, HIV transcription and replication, immune dysfunction, and disease progression compared to HIV+ non-drug users [28-31]. Drug use/abuse has been shown to associate with low CD4+ T cell counts in HIV-infected patients, most likely due to loss of adherence to ART treatment and uncontrolled viremia [32, 33]. The link of drug use/abuse to CD4+ T cell reconstitution in the setting of long-term viral suppression on ART treatment is unknown.

In the current study, we examined plasma anti-CD4 IgGs and their effects on CD4+ T cell recovery in HIV. Here in the setting of ART treatment and long-term viral suppression, we found that drug use and nadir CD4+ T cell counts were independently associated with CD4+ T cell recovery in treated HIV disease after controlling the effects for sex and age. In addition, anti-CD4 autoantibodies from HIV+ drug users mediate CD4+ T cell death. Thus, our results suggest that drug use may play a role in blunted immune restoration in virologically suppressed patients with HIV infection.

Materials and methods

Study subjects

This study was approved by the Institutional Review Board (IRB) for Human Research at the Medical University of South Carolina (MUSC IRB, PRO00062384 and PRO00062196), the clinical core at the University of Alabama at Birmingham (UAB IRB, 880219005), and the CFAR Network of Integrated Clinical Systems (CNIC) study at the UAB (IRB, X020923009). All participants were adult ages above 20 and provided written consent. HIV-negative illicit drug users were recruited from the Department of Psychiatry and Behavioral Sciences at MUSC and HIV-infected subjects were from Infectious Diseases Clinics at MUSC and UAB. To investigate the impact of drug on HIV pathogenesis without confounding by HIV replication and ART adherence, we have included all HIV+ subjects after at least 2 years of virological control by ART treatment, defined by plasma HIV RNA levels below detection limits. We have not excluded patients with transient viremic blips if flanked by undetectable viral levels [11]. HIV+ illicit drug users were on current non-injection cocaine or cannabis but not on prescription drug and other illicit drug uses identified by chart reviews.

Drug and psychiatric disorder diagnostic/descriptive assessment

For HIV-negative drug users, appropriate modules of the Mini-International Neuropsychiatric Interview were used to assess exclusionary psychiatric diagnoses. The M.I.N.I. is a brief structured interview that was designed to assess current Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V) diagnoses using a series of questions in dichotomous format (yes/no) [34]. It is similar in sensitivity, specificity, and inter-rater reliability to other, more lengthy diagnostic interviews, such as the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P) [34, 35]. Because the M.I.N.I. only assesses current diagnoses and a more thorough history of substance use is needed, the substance use module of the Structured Clinical Interview for DSM-V [35] was used for substance use disorder diagnosis as well as to obtain data on initiation and duration of drug use. Substance use in the three months prior to testing was assessed using the Timeline Follow-Back [36]. This is a calendar-based instrument used with specific probes to ascertain detailed information about amounts of substance use. Although generally used in alcohol research, we have successfully modified and used this instrument for other drug use. Drug screens were performed using the onTrak test cup, an *in vitro* diagnostic test for the qualitative detection of drug or drug metabolite in the urine. Results of urine screenings [37, 38] were used to substantiate self-reports of drug use.

Flow cytometry

EDTA-contained blood was used to separate peripheral blood mononuclear cells (PBMC) over a Ficoll-Hypaque cushion (GE, Pittsburgh, PA). Antibodies were incubated with PBMCs at 4°C for 30 min for surface staining. Antibodies were incubated with PBMCs at 4°C for 30 min for surface staining and 30 min for intracellular staining after membrane permeabilization (Fixation/Permeabilization Solution Kit, BD Pharmingen, San Jose, CA). The following fluorochrome-labeled monoclonal antibodies were used: anti-human CD3-

percp, anti-CD4-parcific blue, anti-CD8-APC, annexin V-FITC, and isotype control antibodies. Cells were harvested by BD FACSVerse Flow Cytometer (BD Biosciences) and data were analyzed by FlowJo software (Version 10.0.8).

Plasma level of anti-CD4 IgGs

Development of plasma anti-CD4 IgG detection was published in our previous study [39]. Briefly, human soluble CD4 protein (sCD4, Progenics Tarrytown, NY) was diluted and used to detect anti-CD4 IgG in plasma by ELISA. Microwells were washed and blocked with PBS containing 3% bovine serum albumin (BSA). Plasma was diluted 1:40 in PBS containing 3% BSA. Biotin labeled goat anti-human IgG at 1:5000 dilution and horseradish peroxidase conjugated streptavidin (HRP-Streptavidin) at a 1:1000 dilution were used, and finally 405 nm emission was read within 30 min.

Antibody affinity purification

Methods of antibody purification in plasma were published in our previous study [39]. Briefly, total IgGs were purified from plasma using protein A/G agarose beads following the manufacturer's instruction (Pierce, Pittsburgh, PA), and high affinity anti-CD4 specific IgGs were purified from plasma using NHS Mag Sepharose (GE Healthcare, Wauwatosa, WI) covalently coupled with soluble CD4 protein (sCD4, Progenics Tarrytown, NY; and MyBiosource, San Diego, CA). In the presence of 2M Urea, 1:1 ratio of plasma samples and binding buffer were mixed and incubated at 4°C for 4 h in a column with sCD4 coupled magnetic beads. A magnetic tube rack was used for collection of the unbound fraction. 50 mM Tris/150 mM NaCl and 2M Urea were used to wash the column extensively for high affinity antibody purification. 0.1 M Glycine/HCl buffer and 2M Urea at pH 2.9 were used to elute antigen-specific polyclonal IgG sequentially. Ultra-centrifugal filters (Amicon, EMD Millipore, Massachusetts) were used for concentrating purified IgGs and ELISA was applied to assess their concentrations. Standard curves were generated using the human IgG (ThermoFisher, Rockford, IL) and human monoclonal anti-CD4 antibody Zanolimumab (HuMax-CD4, Genmab). To prepare negative controls, purified anti-CD4 IgG-depleted total IgGs from HIV+ drug users were isolated by sCD4 protein-coupled NHS magnetic beads using NHS Mag Sepharose. A human monoclonal anti-CD4 antibody Zanolimumab was used as a positive control.

ADCC

Method of ADCC was published in our previous study [39]. Briefly, NK cells and CD4+ T cells were isolated from PBMC from aviremic ART-treated HIV+ subjects using NK cell enrichment kit and CD4 cell enrichment kit (StemCell, BC, Canada). The purities of CD4+ T cells and NK cells were above 95% and 94% respectively. NK cells were co-cultured with CD4+ T cells at a 3:1 ratio in 96-well V-bottom plates (Corning, NY) in the presence of anti-CD4 IgGs and controls. Cells were collected and evaluated absolute numbers, apoptosis, and cytotoxicity of CD4+ T cells.

Statistical analysis

All data were analyzed and graphed using Prism software (GraphPad 6.0, La Jolla, CA), SAS (Version 9.3, Cary, NC, USA), or SPSS (Version 23, Chicago, IL, USA). Statistical significance between two groups was determined by the Mann-Whitney test (non-parametric). In the pre-specified hypothesis, we were interested in the comparisons of HIV+ drug users versus HIV+ non-drug users or HIV-negative drug users or healthy controls; therefore, p-values from comparing HIV+ drug users to each of control groups were not adjusted for multiple comparisons [40]. Associations between pairs of continuous variables were analyzed by Spearman Correlation test. A multivariable linear regression model with log transformation of CD4+ T cell counts was used to estimate the effect of drug use after controlling confounders. All tests were 2-sided, and $P < 0.05$ was considered to denote statistical significance.

Results

HIV+ Illicit drug users experience blunted CD4+ T cell recovery despite viral suppression and ART treatment compared to HIV+ non-drug users and HIV-negative drug user controls

First, we have analyzed data from 14 healthy non-drug use controls, 7 HIV-negative illicit drug users, 83 HIV+ non-drug users, and 42 HIV+ illicit drug users with at least 2 years of viral suppression on ART treatment. All data in figure 1 were obtained from MUSC database in the Division of Infectious Diseases. Interestingly, absolute count of peripheral CD4+ T cells (Fig. 1A) was significantly lower but absolute count of peripheral CD8+ T cells (Fig. 1B) was significantly higher in HIV+ illicit drug users compared to HIV+ non-drug users. This result indicates that HIV+ drug users experience a CD4-specific cell decline even with ART treatment.

Both drug use and nadir CD4+ T cell counts is independently associated with CD4+ T cell counts in viral-suppressed HIV+ subject under ART treatment

To define whether drug is an independent factor associated with CD4+ T cell counts in patients, we have assessed a multiple linear regression model (Table 1). CD4+ T cell counts were the outcome and log-transformed due to skewness in their distributions. Because sex [41], age [42], and nadir CD4+ T cell counts [43] have been show to associate with CD4+ T cell counts and HIV disease progression, they were included as covariates. An initial regression model was fit, first controlling for each independent variable separately, and then a final model was fit adjusting for all covariates to evaluate bivariate relationships and unique relationships. Our result shows that sex and age were not independently associated with the outcome; both study group ($P = 0.01$) and nadir CD4+ T cell counts ($P < 0.001$) were independently associated with the outcome. Comparing patients with drug use, CD4+ T cell counts for patients without drug use on average increases by a factor of 1.3 ($p = 0.01$); similarly, per 100 nadir CD4+ T cell counts increase, CD4+ T cells increase by a factor of 1.11 ($p < 0.001$). The results suggest that illicit drug may play a role in CD4+ T cell recovery in virologically controlled HIV disease.

Increased plasma anti-CD4 autoantibody level in aviremic ART-treated HIV+ drug users and its inverse correlation with peripheral CD4+ T cell counts

Next, to study immune perturbation related to drug use and poor CD4+ T cell recovery in viral-suppressed ART-treated HIV+ subjects, we have analyzed plasma level of anti-CD4 IgG and its correlation with CD4+ T cell counts. Notably, HIV+ drug use was associated with increased plasma level of anti-CD4 IgG and decreased CD4+ T cell counts compared to the other three study control groups (Fig. 2A-2B). Furthermore, the plasma level of anti-CD4 IgG was inversely correlated with CD4+ T cell counts only in HIV+ drug users (Fig. 2C) but not in the other three study control groups (Fig. 2C-2D). There were no significant differences in age, race, sex, or duration of ART treatment between the two HIV+ study groups. The median nadir CD4+ T cell counts (cells/ μ L) were 434 (interquartile range, 237-508) and 236 (interquartile range, 149-334) for HIV+ non-drug and drug users respectively ($P = 0.03$).

Significant CD4+ T cell reconstitution following viral-suppressive ART treatment in HIV-infected non-drug users but not in HIV-infected drug users

Furthermore, to determine whether there was a difference in CD4+ T cell recovery following viral-suppressive ART treatment between non-drug users and drug users in HIV disease, we investigated CD4+ T cell counts pre- and post-ART treatment. In deed, we found that CD4+ T cell reconstitution following one and two years of ART treatment was significant in HIV-infected non-drug users but not in HIV-infected drug users (Fig. 2E-2F). This result suggests that drug use associates with CD4+ T cell reconstitution following ART treatment, and this is not due to difference in adherence to medication.

Anti-CD4 autoantibodies from HIV+ drug users mediate CD4+ T cell death through ADCC

To study the effect of anti-CD4 IgGs on CD4+ T cells, we examined whether anti-CD4 IgGs from HIV+ drug users induce ADCC against primary CD4+ T cells. Anti-CD4 IgGs were purified from plasma of HIV+ drug users. NK and CD4+ T cells were isolated from ART-treated HIV+ subjects. Because the plasma levels of anti-CD8 IgGs were very low [39], we used anti-CD4 IgG-depleted total IgGs from HIV+ drug users as a negative control and a human anti-CD4 monoclonal Ab (mAb, zanolimumab) as a positive control for ADCC assays. Detailed methods were described in our recent work [39]. Notably, purified anti-CD4 IgGs from aviremic ART-treated HIV+ drug users induced CD4+ T cell cytolysis (Fig. 3A) and apoptosis (Fig. 3B), while anti-CD4 IgGs from anti-CD4 IgG-depleted total IgGs from ART+ subjects did not mediate ADCC. These results suggest that anti-CD4 IgGs from ART-treated HIV+ drug users are able to induce NK-mediated CD4+ T cell death *in vitro*.

Discussion

In the current study, we found that drug use is associated with poor CD4+ T cell recovery in HIV-infected ART-treated individuals under long-term virologically successful ART treatment.

Patients with incomplete CD4+ T cell reconstitution have been shown to exhibit a sustained subclinical immune deficiency distinguishable from patients with CD4+ T cell counts similar to healthy controls, and experience increased complications, morbidity and mortality due to cardiovascular, liver and kidney diseases [44-46]. Lack of increase in CD4+ T cell count despite virologically suppressive ART indicates need for alternative therapeutic strategies. Our study suggests that drug use is associated with blunted CD4+ T cell recovery in ART-treated HIV disease.

Drug use may increase viral load, accelerate HIV progression and increase mortality even among ART adherent patients [33]. It is challenging to study the effect of illicit drug on HIV pathogenesis due to serious confounding factors. Notably, drug use/abuse is often related to decreased adherence to ART treatment that largely complicates a direct link between drug use and HIV disease progression [47, 48]. Other drug-related factors such as single drug use or concurrent use of multiple illicit substances, and history and route of drug use, are confounding for defining the mechanism by which illicit substances exacerbate disease progression [47, 48]. For example, increased T cell activation and substance use/abuse are associated with incomplete immune restoration in HIV disease; however, these may be largely due to uncontrolled HIV replication and low adherence to ART treatment in patients with drug use/abuse [49-53]. Nonetheless, results in animal *in vivo* and in human *in vitro* suggest that illicit drugs (e.g., cocaine, marijuana, opiates) affect HIV viral replication [54-56]. In addition, high dose (200-1000 mg/L) cocaine has been shown to induce CD4+ T cell apoptosis *in vitro* [57], suggesting that drug may directly impair immune function and contribute to loss of virologic control. In the current study, we found that drug is independently associated with CD4+ T cell reconstitution in treated HIV disease.

Both drugs and HIV target the central nervous system, accelerate neuronal and cognitive impairment, and induce disruption of the blood brain barrier and increase its permeability [58]. Illicit drugs have been shown to associate with increased systemic inflammation [59, 60], which may result from cellular activation and oxidative stress [61, 62], and induction of gut microbial translocation [21, 22, 63, 64]. Studies from our group and others indicate that HIV infection is associated with a permeable gut, increased microbial translocation, and increased CD4+ T cell apoptosis and inflammation [11, 65-67]. Moreover, HIV Tat protein and gp120 directly reduce tight junction expression on epithelial cells *in vitro* [68, 69]. However, the exact mechanism of drug mediated CD4+ T cell decline in HIV pathogenesis especially controlling for HIV replication and ART adherence is largely unknown.

One limitation of the current study is the small sample size and including both cocaine and cannabis users. We did not find a difference in all analysis between cocaine and cannabis users. The other limitation is that the methods of illicit drug user enrollment were different from HIV-negative and HIV-infected subjects. HIV-infected illicit drug users were identified based on self-reports without drug screens. This limitation may account for some immune functional differences between HIV-negative and HIV-infected illicit drug users. In summary, we found ART+ aviremic HIV+ drug users have increased plasma anti-CD4 autoantibodies, which may account for decreased CD4+ T cell counts in these patients after ART treatment.

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Reference:

- [1]. Donnell D; Baeten JM; Kiarie J; Thomas KK; Stevens W; Cohen CR; McIntyre J; Lingappa JR; Celum C; Team, P.i.P.H.H.T.S., Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. *The Lancet*, 2010, 375, (9731), 2092–2098.
- [2]. Mocroft A; Phillips AN; Gatell J; Ledergerber B; Fisher M; Clumeck N; Losso M; Lazzarin A; Fatkenheuer G; Lundgren J, Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. *The Lancet*, 2007, 370, (9585), 407–413.
- [3]. Battegay M; Nüesch R; Hirschel B; Kaufmann GR, Immunological recovery and antiretroviral therapy in HIV-1 infection. *The Lancet infectious diseases*, 2006, 6, (5), 280–287. [PubMed: 16631548]
- [4]. Kaufmann GR; Furrer H; Ledergerber B; Perrin L; Opravil M; Vernazza P; Cavassini M; Bernasconi E; Rickenbach M; Hirschel B, Characteristics, Determinants, and Clinical Relevance of CD4 T Cell Recovery to < 500 Cells/μL in HIV Type 1—Infected Individuals Receiving Potent Antiretroviral Therapy. *Clinical infectious diseases*, 2005, 41, (3), 361–372. [PubMed: 16007534]
- [5]. Guihot A; Bourgarit A; Carcelain G; Autran B, Immune reconstitution after a decade of combined antiretroviral therapies for human immunodeficiency virus. *Trends Immunol*, 2011, 32, (3), 131–137. [PubMed: 21317040]
- [6]. Baker JV; Peng G; Rapkin J; Abrams DI; Silverberg MJ; MacArthur RD; Cavert WP; Henry WK; Neaton JD, CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. *AIDS (London, England)*, 2008, 22, (7), 841.
- [7]. El-Sadr W; Lundgren JD; Neaton J; Gordin F; Abrams D; Arduino R; Babiker A; Burman W; Clumeck N; Cohen C, CD4+ count-guided interruption of antiretroviral treatment. *New Engl J Med*, 2006, 355, (22), 2283–2296. [PubMed: 17135583]
- [8]. Silverberg MJ; Neuhaus J; Bower M; Gey D; Hatzakis A; Henry K; Hidalgo J; Lourtou L; Neaton JD; Tambussi G, Risk of cancers during interrupted antiretroviral therapy in the SMART study. *Aids*, 2007, 21, (14), 1957–1963. [PubMed: 17721103]
- [9]. Lapadula G; Cozzi-Lepri A; Marchetti G; Antinori A; Chiodera A; Nicastri E; Parruti G; Galli M; Gori A; Monforte A.d.A., Risk of clinical progression among patients with immunological nonresponse despite virological suppression after combination antiretroviral treatment. *Aids*, 2013, 27, (5), 769–779. [PubMed: 23719349]
- [10]. Group, D.C.o.A.E.o.A.-H.D.S., HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malignancies. *AIDS (London, England)*, 2008, 22, (16), 2143.
- [11]. Lederman MM; Calabrese L; Funderburg NT; Clagett B; Medvik K; Bonilla H; Gripshover B; Salata RA; Taege A; Lisgaris M; McComsey GA; Kirchner E; Baum J; Shive C; Asaad R; Kalayjian RC; Sieg SF; Rodriguez B, Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis*, 2011, 204, (8), 1217–1226. [PubMed: 21917895]
- [12]. Hunt PW; Brenchley J; Sinclair E; McCune JM; Roland M; Page-Shafer K; Hsue P; Emu B; Krone M; Lampiris H; Douek D; Martin JN; Deeks SG, Relationship between T cell activation

- and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis*, 2008, 197, (1), 126–133. [PubMed: 18171295]
- [13]. Doitsh G; Cavrois M; Lassen KG; Zepeda O; Yang Z; Santiago ML; Hebbeler AM; Greene WC, Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. *Cell*, 2010, 143, (5), 789–801. [PubMed: 21111238]
- [14]. Grossman Z; Meier-Schellersheim M; Paul WE; Picker LJ, Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med*, 2006, 12, (3), 289–295. [PubMed: 16520776]
- [15]. Day CL; Kaufmann DE; Kiepiela P; Brown JA; Moodley ES; Reddy S; Mackey EW; Miller JD; Leslie AJ; DePierres C; Mncube Z; Duraiswamy J; Zhu B; Eichbaum Q; Altfeld M; Wherry EJ; Coovadia HM; Goulder PJ; Klennerman P; Ahmed R; Freeman GJ; Walker BD, PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*, 2006, 443, (7109), 350–354. [PubMed: 16921384]
- [16]. Doitsh G; Galloway NL; Geng X; Yang Z; Monroe KM; Zepeda O; Hunt PW; Hatano H; Sowinski S; Munoz-Arias I; Greene WC, Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*, 2014, 505, (7484), 509–514. [PubMed: 24356306]
- [17]. Mattapallil JJ; Douek DC; Hill B; Nishimura Y; Martin M; Roederer M, Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature*, 2005, 434, (7037), 1093–1097. [PubMed: 15793563]
- [18]. Okoye AA; Picker LJ, CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol Rev*, 2013, 254, (1), 54–64. [PubMed: 23772614]
- [19]. Kuwata T; Nishimura Y; Whitted S; Ourmanov I; Brown CR; Dang Q; Buckler-White A; Iyengar R; Brenchley JM; Hirsch VM, Association of progressive CD4(+) T cell decline in SIV infection with the induction of autoreactive antibodies. *PLoS pathogens*, 2009, 5, (4), e1000372. [PubMed: 19360097]
- [20]. Brenchley JM; Price DA; Schacker TW; Asher TE; Silvestri G; Rao S; Kazzaz Z; Bornstein E; Lambotte O; Altmann D, Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature medicine*, 2006, 12, (12), 1365–1371.
- [21]. Meng J; Yu H; Ma J; Wang J; Banerjee S; Charboneau R; Barke RA; Roy S, Morphine induces bacterial translocation in mice by compromising intestinal barrier function in a TLR-dependent manner. *PLoS One*, 2013, 8, (1), e54040. [PubMed: 23349783]
- [22]. Banerjee S; Sindberg G; Wang F; Meng J; Sharma U; Zhang L; Dauer P; Chen C; Dalluge J; Johnson T; Roy S, Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systemic inflammation. *Mucosal Immunol*, 2016.
- [23]. Anthony IC; Arango JC; Stephens B; Simmonds P; Bell JE, The effects of illicit drugs on the HIV infected brain. *Front Biosci*, 2008, 13, 1294–1307. [PubMed: 17981630]
- [24]. Bell JE; Brettell RP; Chiswick A; Simmonds P, HIV encephalitis, proviral load and dementia in drug users and homosexuals with AIDS. Effect of neocortical involvement. *Brain*, 1998, 121 (Pt 11), 2043–2052. [PubMed: 9827765]
- [25]. Silverstein PS; Shah A; Gupte R; Liu X; Piepho RW; Kumar S; Kumar A, Methamphetamine toxicity and its implications during HIV-1 infection. *J Neurovirol*, 2011, 17, (5), 401–415. [PubMed: 21786077]
- [26]. Gaskill PJ; Calderon TM; Coley JS; Berman JW, Drug induced increases in CNS dopamine alter monocyte, macrophage and T cell functions: implications for HAND. *J Neuroimmune Pharmacol*, 2013, 8, (3), 621–642. [PubMed: 23456305]
- [27]. Meyer VJ; Little DM; Fitzgerald DA; Sundermann EE; Rubin LH; Martin EM; Weber KM; Cohen MH; Maki PM, Crack cocaine use impairs anterior cingulate and prefrontal cortex function in women with HIV infection. *J Neurovirol*, 2014, 20, (4), 352–361. [PubMed: 24760360]
- [28]. Baum MK; Campa A; Page JB; Lai S; Tsalaile L; Martinez SS; Burns P; Williams O; Li Y; van Widenfelt E; Bussmann H; Sikhulele M; Makhema J; Essex M; Marlink R, Recruitment, Follow-Up and Characteristics of HIV Infected Adults who Use Illicit Drugs in Southern Africa. *J Drug Abuse*, 2015, 1, (1).

- [29]. Tyagi M; Weber J; Bukrinsky M; Simon GL, The effects of cocaine on HIV transcription. *J Neurovirol*, 2016, 22, (3), 261–274. [PubMed: 26572787]
- [30]. Ellis RJ; Childers ME; Cherner M; Lazzaretto D; Letendre S; Grant I; Group H.I.V.N.R.C., Increased human immunodeficiency virus loads in active methamphetamine users are explained by reduced effectiveness of antiretroviral therapy. *J Infect Dis*, 2003, 188, (12), 1820–1826. [PubMed: 14673760]
- [31]. Fairbairn N; Kerr T; Milloy MJ; Zhang R; Montaner J; Wood E, Crystal methamphetamine injection predicts slower HIV RNA suppression among injection drug users. *Addict Behav*, 2011, 36, (7), 762–763. [PubMed: 21396784]
- [32]. Cook JA; Grey DD; Burke-Miller JK; Cohen MH; Vlahov D; Kapadia F; Wilson TE; Cook R; Schwartz RM; Golub ET; Anastos K; Ponath C; Goparaju L; Levine AM, Illicit drug use, depression and their association with highly active antiretroviral therapy in HIV-positive women. *Drug Alcohol Depend*, 2007, 89, (1), 74–81. [PubMed: 17291696]
- [33]. Passaro RC; Pandhare J; Qian HZ; Dash C, The Complex Interaction Between Methamphetamine Abuse and HIV-1 Pathogenesis. *J Neuroimmune Pharmacol*, 2015, 10, (3), 477–486. [PubMed: 25850893]
- [34]. Sheehan DV LY, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC, The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*, 1998, 59 (suppl 20:), 20–33.
- [35]. First MB, Spitzer RL, Gibbon M, & Williams JBW, Structured clinical interview for DSM-IV-TR Axis I disorders, research version, patient edition. (SCID-I/P) New York: Biometrics Research, New York State Psychiatric Institute, 2002.
- [36]. Toneatto A; Sobell LC; Sobell MB, Gender issues in the treatment of abusers of alcohol, nicotine, and other drugs. *J Subst Abuse*, 1992, 4, (2), 209–218. [PubMed: 1504644]
- [37]. Skinner HA, The drug abuse screening test. *Addict Behav*, 1982, 7, (4), 363–371. [PubMed: 7183189]
- [38]. Gavin DR; Ross HE; Skinner HA, Diagnostic validity of the drug abuse screening test in the assessment of DSM-III drug disorders. *Br J Addict*, 1989, 84, (3), 301–307. [PubMed: 2650770]
- [39]. Luo ZW, L. Z, Martin L, Wan Z, Meissner EG, Espinosa E, Wu H, Yu XC, Fu PF, Westerink MAJ, Kilby JM, Wu J, Huang L, Heath SL, Li ZH, Jiang W, Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy. *Journal of Infectious Diseases*, 2017, Epub ahead of print.
- [40]. Rothman KJ, No adjustments are needed for multiple comparisons. *Epidemiology*, 1990, 1, (1), 43–46. [PubMed: 2081237]
- [41]. Meier A; Chang JJ; Chan ES; Pollard RB; Sidhu HK; Kulkarni S; Wen TF; Lindsay RJ; Orellana L; Mildvan D; Bazner S; Streeck H; Alter G; Lifson JD; Carrington M; Bosch RJ; Robbins GK; Altfeld M, Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med*, 2009, 15, (8), 955–959. [PubMed: 19597505]
- [42]. Means AR; Risher KA; Ujeneza EL; Maposa I; Nondi J; Bellan SE, Impact of Age and Sex on CD4+ Cell Count Trajectories following Treatment Initiation: An Analysis of the Tanzanian HIV Treatment Database. *PLoS One*, 2016, 11, (10), e0164148. [PubMed: 27716818]
- [43]. Negrodo E; Massanella M; Puig J; Perez-Alvarez N; Gallego-Escuredo JM; Villarroya J; Villarroya F; Molto J; Santos JR; Clotet B; Blanco J, Nadir CD4 T cell count as predictor and high CD4 T cell intrinsic apoptosis as final mechanism of poor CD4 T cell recovery in virologically suppressed HIV-infected patients: clinical implications. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 2010, 50, (9), 1300–1308. [PubMed: 20367229]
- [44]. Baker JV; Peng G; Rapkin J; Abrams DI; Silverberg MJ; MacArthur RD; Cavert WP; Henry WK; Neaton JD; Terry Beinr Community Programs for Clinical Research on, A., CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. *Aids*, 2008, 22, (7), 841–848. [PubMed: 18427202]
- [45]. Lewden C; Chene G; Morlat P; Raffi F; Dupon M; Dellamonica P; Pellegrin JL; Katlama C; Dabis F; Leport C; Agence Nationale de Recherches sur le Sida et les Hepatitis Virales, C.O.A.-

C.S.G.; Agence Nationale de Recherches sur le Sida et les Hepatites Virales, C.O.A.S.G., HIV-infected adults with a CD4 cell count greater than 500 cells/mm³ on long-term combination antiretroviral therapy reach same mortality rates as the general population. *J Acquir Immune Defic Syndr*, 2007, 46, (1), 72–77. [PubMed: 17621240]

- [46]. Gutierrez F; Padilla S; Masia M; Iribarren JA; Moreno S; Viciano P; Hernandez-Quero J; Aleman R; Vidal F; Salavert M; Blanco JR; Leal M; Dronda F; Perez Hoyos S; del Amo J; Co RM, Patients' characteristics and clinical implications of suboptimal CD4 T-cell gains after 1 year of successful antiretroviral therapy. *Current HIV research*, 2008, 6, (2), 100–107. [PubMed: 18336257]
- [47]. Parsons JT; Kowalczyk WJ; Botsko M; Tomassilli J; Golub SA, Aggregate versus day level association between methamphetamine use and HIV medication non-adherence among gay and bisexual men. *AIDS Behav*, 2013, 17, (4), 1478–1487. [PubMed: 23553345]
- [48]. Marquez C; Mitchell SJ; Hare CB; John M; Klausner JD, Methamphetamine use, sexual activity, patient-provider communication, and medication adherence among HIV-infected patients in care, San Francisco 2004–2006. *AIDS Care*, 2009, 21, (5), 575–582. [PubMed: 19444665]
- [49]. Valdez H; Connick E; Smith KY; Lederman MM; Bosch RJ; Kim RS; St Clair M; Kuritzkes DR; Kessler H; Fox L; Blanchard-Vargas M; Landay A; Team ACTGP, Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS*, 2002, 16, (14), 1859–1866. [PubMed: 12351945]
- [50]. Hunt PW; Martin JN; Sinclair E; Brecht B; Hagos E; Lampiris H; Deeks SG, T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *Journal of Infectious Diseases*, 2003, 187, (10), 1534–1543. [PubMed: 12721933]
- [51]. Landay A; Benning L; Bremer J; Weiser B; Burger H; Nowicki M; Kovacs A, Correlates of immune activation marker changes in human immunodeficiency virus (HIV)-seropositive and high-risk HIV-seronegative women who use illicit drugs. *J Infect Dis*, 2003, 188, (2), 209–218. [PubMed: 12854075]
- [52]. Cescon A; Chan K; Raboud JM; Burchell AN; Forrest JI; Klein MB; Loutfy MR; Machouf N; Montaner JS; Tsoukas C; Hogg RS; Cooper C; Collaboration C, Significant differences in clinical outcomes between HIV-hepatitis C virus coinfecting individuals with and without injection drug use history. *AIDS*, 2014, 28, (1), 121–127. [PubMed: 24413263]
- [53]. Engsig FN; Zangerle R; Katsarou O; Dabis F; Reiss P; Gill J; Porter K; Sabin C; Riordan A; Fatkenheuer G; Gutierrez F; Raffi F; Kirk O; Mary-Krause M; Stephan C; de Olalla PG; Guest J; Samji H; Castagna A; d'Arminio Monforte A; Skaletz-Rorowski A; Ramos J; Lapadula G; Mussini C; Force L; Meyer L; Lampe F; Boufassa E; Bucher HC; De Wit S; Burkholder GA; Teira R; Justice AC; Sterling TR; H MC; Gerstoft J; Grarup J; May M; Chene G; Ingle SM; Sterne J; Obel N; Antiretroviral Therapy Cohort, C.; the Collaboration of Observational, H.I.V.E.R.E.i.E., Long-term mortality in HIV-positive individuals virally suppressed for >3 years with incomplete CD4 recovery. *Clin Infect Dis*, 2014, 58, (9), 1312–1321. [PubMed: 24457342]
- [54]. Wang X; Ho WZ, Drugs of abuse and HIV infection/replication: implications for mother-fetus transmission. *Life Sci*, 2011, 88, (21-22), 972–979.
- [55]. Mantri CK; Mantri JV; Pandhare J; Dash C, Methamphetamine inhibits HIV-1 replication in CD4+ T cells by modulating anti-HIV-1 miRNA expression. *Am J Pathol*, 2014, 184, (1), 92–100. [PubMed: 24434277]
- [56]. Suzuki S; Carlos MP; Chuang LF; Torres JV; Doi RH; Chuang RY, Methadone induces CCR5 and promotes AIDS virus infection. *FEBS Lett*, 2002, 519, (1-3), 173–177. [PubMed: 12023039]
- [57]. Pandhare J; Addai AB; Mantri CK; Hager C; Smith RM; Barnett L; Villalta F; Kalams SA; Dash C, Cocaine enhances HIV-1-induced CD4(+) T-cell apoptosis: implications in disease progression in cocaine-abusing HIV-1 patients. *Am J Pathol*, 2014, 184, (4), 927–936. [PubMed: 24486327]
- [58]. Mahajan SD; Aalinkel R; Sykes DE; Reynolds JL; Bindukumar B; Adal A; Qi M; Toh J; Xu G; Prasad PN; Schwartz SA, Methamphetamine alters blood brain barrier permeability via the modulation of tight junction expression: Implication for HIV-1 neuropathogenesis in the context of drug abuse. *Brain Res*, 2008, 1203, 133–148. [PubMed: 18329007]

- [59]. Ahmed SH; Lutjens R; van der Stap LD; Lekic D; Romano-Spica V; Morales M; Koob GF; Repunte-Canonigo V; Sanna PP, Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction. *Proc Natl Acad Sci U S A*, 2005, 102, (32), 11533–11538. [PubMed: 16076954]
- [60]. Piechota M; Korostynski M; Solecki W; Gieryk A; Slezak M; Bilecki W; Ziolkowska B; Kostrzewa E; Cymmerman I; Swiech L; Jaworski J; Przewlocki R, The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. *Genome Biol*, 2010, 11, (5), R48. [PubMed: 20459597]
- [61]. Dalvi P; Wang K; Mermis J; Zeng R; Sanderson M; Johnson S; Dai Y; Sharma E; Ladner AO; Dhillon NK, HIV-1/cocaine induced oxidative stress disrupts tight junction protein-1 in human pulmonary microvascular endothelial cells: role of Ras/ERK1/2 pathway. *PLoS One*, 2014, 9, (1), e85246. [PubMed: 24409324]
- [62]. Poon HF; Abdullah L; Mullan MA; Mullan MJ; Crawford FC, Cocaine-induced oxidative stress precedes cell death in human neuronal progenitor cells. *Neurochem Int*, 2007, 50, (1), 69–73. [PubMed: 16956698]
- [63]. Sindberg GM; Sharma U; Banerjee S; Anand V; Dutta R; Gu CJ; Volsky DJ; Roy S, An infectious murine model for studying the systemic effects of opioids on early HIV pathogenesis in the gut. *J Neuroimmune Pharmacol*, 2015, 10, (1), 74–87. [PubMed: 25502600]
- [64]. Meng J; Sindberg GM; Roy S, Disruption of gut homeostasis by opioids accelerates HIV disease progression. *Front Microbiol*, 2015, 6, 643. [PubMed: 26167159]
- [65]. Funderburg N; Luciano AA; Jiang W; Rodriguez B; Sieg SF; Lederman MM, Toll-like receptor ligands induce human T cell activation and death, a model for HIV pathogenesis. *PLoS One*, 2008, 3, (4), e1915. [PubMed: 18382686]
- [66]. Jiang W; Lederman MM; Hunt P; Sieg SF; Haley K; Rodriguez B; Landay A; Martin J; Sinclair E; Asher AI; Deeks SG; Douek DC; Brechley JM, Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis*, 2009, 199, (8), 1177–1185. [PubMed: 19265479]
- [67]. Jiang W; Younes SA; Funderburg NT; Mudd JC; Espinosa E; Davenport MP; Babineau DC; Sieg SF; Lederman MM, Cycling memory CD4+ T cells in HIV disease have a diverse T cell receptor repertoire and a phenotype consistent with bystander activation. *Journal of virology*, 2014, 88, (10), 5369–5380. [PubMed: 24522925]
- [68]. Bai L; Zhang Z; Zhang H; Li X; Yu Q; Lin H; Yang W, HIV-1 Tat protein alter the tight junction integrity and function of retinal pigment epithelium: an in vitro study. *BMC Infect Dis*, 2008, 8, 77. [PubMed: 18538010]
- [69]. Nazli A; Chan O; Dobson-Belaire WN; Ouellet M; Tremblay MJ; Gray-Owen SD; Arsenault AL; Kaushic C, Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. *PLoS Pathog*, 2010, 6, (4), e1000852. [PubMed: 20386714]

Figure 1.

HIV+ drug users have decreased CD4+ T cell counts but increased CD8+ T cell counts after viral suppressive ART treatment. The median CD4+ T cell counts (A) and the median CD8+ T cell counts (B) were analyzed in fresh blood samples by flow cytometry in 14 healthy controls (HIV-/ND), 7 HIV-negative drug users (HIV-/D), 83 HIV+ non-drug users (HIV+/ND), and 42 HIV+ drug users (HIV+/D).

Figure 2.

Increased plasma level of anti-CD4 IgG in HIV+ drug users and its inverse correlation with peripheral CD4+ T cell counts. Anti-CD4 IgG was analyzed in plasma by ELISA. (A) Plasma level of anti-CD4 IgG (A) and peripheral CD4+ T cell counts (B) in the four study groups. Correlations between plasma anti-CD4 IgG level and CD4+ T cell counts in HIV+ subjects (C) and HIV-negative subjects (D). CD4+ T cell counts pre-ART and 1 and 2 years of post-ART in HIV+ non-drug users (HIV+/ND, E) and HIV+ drug users (HIV+/D, F).

Table 1.

Results of multivariable linear regression on log scale of CD4+ T cell counts

Parameter	Estimate of coefficient	Standard Error	T-statistic	p-value
Intercept	6.14	0.16	37.93	< 0.0001
Sex (Female vs. Male)	−0.009	0.08	−0.11	0.91
Age (per year increase)	−0.003	0.003	−1.05	0.3
Drug vs Non-drug	−0.26	0.1	−2.58	0.01
Nadir CD4 (per nadir CD4 increase)	0.001	0.0002	8.06	< 0.0001