Cocaine Alters Cytokine Profiles in HIV-1-Infected African American Individuals in the DrexelMed HIV/AIDS Genetic Analysis Cohort

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Abstract

Background—This study evaluated the relationship between illicit drug use and HIV-1 disease severity in HIV-1-infected patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort. Since, cocaine is known to have immunomodulatory effects, the cytokine profiles of preferential nonusers, cocaine users, and multidrug users were analyzed to understand the effects of cocaine on cytokine modulation and HIV-1 disease severity.

Methods—Patients within the cohort were assessed approximately every 6 months for HIV-1 clinical markers and for history of illicit drug, alcohol, and tobacco use. The Luminex human cytokine 30-plex panel was used for cytokine quantitation. Analysis was performed using a newly developed biostatistical model.

Results—Substance abuse was common within the cohort. Utilizing the drug screens at the time of each visit, the subjects in the cohort were categorized as preferential nonusers, cocaine users, or multidrug users. The overall health of the nonuser population was better than that of the cocaine users, with peak and current viral loads in nonusers substantially lower than those in cocaine and

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multidrug users. Among the 30 cytokines investigated, differential levels were established within the 3 populations. The T-helper 2 cytokines, interleukin-4 and -10, known to play a critical role during HIV-1 infection, were positively associated with increasing cocaine use. Clinical parameters such as latest viral load, CD4+ T-cell counts, and CD4:CD8 ratio were also significantly associated with cocaine use, depending on the statistical model used.

Conclusions—Based on these assessments, cocaine use appears to be associated with more severe HIV-1 disease.

Keywords

HIV; cocaine; cytokines; drug use; interleukin-4; interleukin-10

INTRODUCTION

Illicit drug use is one of the most prevalent and major risk factors for HIV transmission.[1] Intravenous drug use is the predominant reason for the increased transmission of HIV-1. However, other routes of cocaine administration (inhalation, snorting, smoking, and injection) are also correlated with an almost 3-fold increased association with HIV-1 transmission[2] and with accelerated progression to AIDS.[3] Cocaine has been designated as an immunomodulator based on studies showing that acute exposure can alter the expression of proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), in monocytes of cocaine-dependent individuals and that chronic exposure may result in greater suppression.[4] Cocaine exerts its effects through the dopamine receptors and the sigma-1 receptor, which is involved in altered immune function and has been identified on peripheral blood mononuclear cells (PBMCs) and cells within the central nervous system.[5,6] Studies in murine peritoneal macrophages treated acutely with selected dosages of cocaine have also shown a suppression of immunological parameters such as IL-1 and TNF-α.[7]

Based on these studies, cocaine has been proposed to disrupt the homeostatic balance between type I and type II helper T cytokines (Th1 and Th2, respectively).[6] CD4+ T-helper cells are the major sources of cytokines and chemokines.[8] Th1 cells produce proinflammatory cytokines such as IL-1, IL-2, IL-6, TNF-α, interferon-γ (IFN-γ), IL-12, and IL-23, and Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13.[9,8] The balance between Th1 and Th2 cytokines plays an influential role in many pathological processes.[8] Cytokines and chemokines play an important role during viral infection in both clearance of the virus and pathogenesis of the infection.[10] Responses from Th1 cells are associated with cell-mediated immunity, whereas responses from Th2 cells are associated with humoral immune responses. Both subsets of cells have distinct profiles of cytokines that are activated by different immune responses.[11] IL-2 and IFN-γ, along with IL-1β and TNF-α, share proinflammatory properties and play an important role in the pathogenesis of autoimmune diseases and infections.[12]

Several cohort studies of HIV-1-infected individuals, have indicated that use of cocaine is associated with decreased adherence to antiretroviral therapy (ART) and with accelerated disease progression,[1,15,16,17] demonstrating that the results from in vitro studies could be corroborated with in vivo studies. In a 6-month cohort study examining adherence to ART

among 85 HIV-1-positive individuals, active cocaine use was significantly associated with lower adherence to therapeutic regimens. Adherence declined 41% among active cocaine users, and the decline was associated with failure to maintain viral suppression.[15] Another prospective, 30-month clinical study enrolled 222 HIV-1-positive drug-abusing individuals. Crack cocaine users were twice as likely to have a decrease in their CD4+ T-cell count below 200 cells/μl, as compared with individuals abusing other drugs such as marijuana, heroin, and alcohol (the study controlled for use of highly active antiretroviral therapy [HAART]). In addition to the observed association with CD4+ T-cell count, crack cocaine was significantly associated with a higher level of HIV-1 RNA.[18] Another retrospective cohort study showed similar results for crack cocaine abusers and also found that crack cocaine abusers developed more AIDS-defining illnesses, such as bacterial pneumonia, pneumocystosis, histoplasmosis, and cerebral toxoplasmosis.[19]

Several cohort studies have shown the impact of cocaine on HIV-1 infection and selected clinical parameters. However, fewer studies have shown correlations between cocaine use and cytokine profiles or immunopathogenesis in HIV-1-infected individuals. One of the few cohort studies analyzed a plasma biomarker signature of immune activation, comparing 57 HIV-1-positive patients who were on combination antiretroviral therapy with 29 HIV-1-negative individuals.[20] About 55% of the HIV-1-infected individuals were abusing cocaine, some of whom were simultaneously abusing opiates. Given this observation, a small number of individuals were abusing cocaine alone; however, drug abuse was determined by self-reporting or urine toxicology and documented longitudinal histories of drug abuse were lacking.[20] A cluster of viremic cocaine users were found to have elevated levels of soluble IL-2R (sIL-2R), a cytokine that can promote the growth and proliferation of T cells.[20] sIL-2R has been shown to be increased in HIV-1-seropositive individuals and a predictor of progression of HIV-1 infection to AIDS; it correlates with response to therapy and remains unaffected by the presence of opportunistic infections, as previously reviewed.[21] In addition to sIL-2R, cytokines that were elevated in viremic as compared with viremic noncocaine users included CXCL9, CXCL10 (IFN-γ-induced protein 10), and CCL4 (macrophage inflammatory protein-1β).[20] These findings raise the likelihood that cocaine is responsible for the deregulation of the immune response.

Though there are many HIV-1 cohort studies, very few of them monitor subjects' use of illicit or recreational drugs, test subjects systematically and longitudinally for illicit drug use, and analyze the results accordingly. Determining the effects of cocaine use in HIV-1-infected individuals by comparing a non–drug using population with a population of individuals preferentially abusing cocaine is an immediate priority. To this end, we have established the DREXELMED HIV/AIDS Genetic Analysis Cohort assessing subjects' past and present drug, alcohol, and tobacco histories in order to more accurately assess the impact of single substances such as cocaine on the severity of HIV-1 disease in the absence of overt polydrug use.

MATERIALS AND METHODS

Patient Recruitment, Clinical Data, and Sample Collection

Patients in the DREXELMED HIV/AIDS Genetic Analysis Cohort have been recruited from the Partnership Comprehensive Care Practice of the Division of Infectious Diseases and HIV Medicine at Drexel University College of Medicine, which is located in center city Philadelphia, Pennsylvania, as previously described.[22] The study was approved by the Drexel University College of Medicine Institutional Review Board under protocol 16311 (Brian Wigdahl, principal investigator) in compliance with Helsinki Declaration of 1975 as revised in 2000. All participants were required to provide informed consent. Once a subject was enrolled, an anonymous identifier was assigned and the patient's clinical data were collected as previously described.[22] Blood samples were drawn from each patient at the initial visit and at each return visit (approximately every 6 months but at least one recall per year) to allow for longitudinal study. Patients were considered preferential nonusers (PN) if they tested negative for all drugs at all visits and never admitted to drug use. Preferential cocaine users (PCo) were patients who tested positive for only cocaine at all visits; patients were dropped from consideration if they tested negative for their preferential drug on two consecutive visits or positive for a different drug at any any visit. Preferential users of cannabinoids (PCa) and benzodiazepines (PBe) were also identified using the same parameters as were used for the PCo group. MDUs had to test positive for the same combination of drugs with identical requirements as the PCo group. Patients were classified into drug-user groups based solely on drug testing results; their verbal admissions or denials were not considered.

Patient Drug Screening

Blood samples from each patient's first and every subsequent visit were screened using a standard 7-drug profile drug screen (LabCorp., Burlington, NC), which included amphetamines (amphetamine and methamphetamine); barbiturates (amobarbital, butalbital, pentobarbital, phenobarbital, and secobarbital); benzodiazepines (desalkylflurazepam, flurazepam, and diazepam); cannabinoids (tetrahydrocannabinol and tetrahydrocannabinolic acid); cocaine (cocaine and benzoylecgonine); opiates (codeine and morphine); and phenylcyclidine.

PBMC Isolation and Plasma Sample Storage

Three purple-top blood collection tubes (Vacutainer; Becton, Dickinson, Franklin Lakes, NJ) containing EDTA were used to collect 50 ml of whole blood from patients for plasma and PBMC isolation as described previously.[22,23]

Luminex 30-Plex Assay

The Human Cytokine 30-plex panel for the Luminex platform (Life Technologies, Grand Island, NY) was used to quantify the indicated cytokines, chemokines, and growth factors present in the plasma samples. The multiplex immunoassay was performed as described by the manufacturer and the plate was read on the Luminex 200 system (Luminex Corp.,

Austin, Texas). Each multiplex immunoassay was performed twice in duplicate, giving 4 values for each cytokine concentration.

Biostatistical Analysis

To begin the experimental analysis, all values that fell off of the standard curve were discarded (5%). Subsequently, all remaining raw cytokine values were quantile transformed for each array using the *normalize.quantiles* function in the Bioconductor package.[24] The difference in each cytokine between drug-user groups was examined using the categorical contribution model (CCM) and the dosage response was tested using the weighted linear contribution model (WLCM). Both models were based on a linear mixed-effects model and included terms for age, gender, HAART status, and hepatitis C virus (HCV coinfection) as confounding variables. Age was considered as a linear variable with the age range of participants between 20 and 71 years. whereas gender, HAART status (continuous, discontinuous, and naïve), and HCV coinfection were treated as categorical variables.

In CCM, patients were grouped into nonusers, single-drug users, and multidrug users (MDU) and dummy variables for each category were included in the model. Any patient who did not fall into these categories was excluded from this section of the analysis.

The algorithm used for the CCM model was as follows: Cytokine = κ (age) + κ (gender) + κ (HAART) + κ (HCV) + κ (logviralload) + κ (druggroup).

The WLCM attempts to model the effect of drug use on the dependent variable by considering each drug as a linear contributor. Data from all patients with results from the cytokine analysis were used to build the WLCM model, regardless of their drug use. This approach provided a methodology to analyze patients with varying levels usage of cocaine and other drugs. The inclusion of patients using only cannabinoids or benzodiazepines allows the algorithm to estimate the effect of cocaine within a multidrug use scenario.

The algorithm for the WLCM model is as follows: Cytokine = κ (age) + κ (gender) + κ (HAART) + κ (HCV) + κ (logviralload) + κ (cocaine) + κ (cannabinoid) + κ (benzodiazepine).

Within the WLCM model, three different methods were constructed to represent the effects of drug use. The first method involved using the positive/negative results of the drug test at the sampled visit as binary variables (termed the AT-VISIT analysis). The second method involved using the fraction of positive tests for each drug up to and including the sampled visit as linear variables (termed the UPTO-VISIT analysis). The third method used the fraction of positive tests for each drug at all visits for a particular patient as linear variables (termed the ALL-VISITS analysis; VISIT is denoted as the visit of the patient at which the plasma sample was used for the Luminex assay).

Statistical analysis was performed using R2.15.1 (The R Foundation for Statistical Computing); p   0.05 was considered significant. Multiple testing comparison was performed using the Benjamini-Hochberg correction; q   0.05.

RESULTS

DrexelMed HIV/AIDS Genetic Analysis Cohort Demographics

At the time of this report, the $D_{REXEL}M_{ED}$ HIV/AIDS Genetic Analysis Cohort was comprised of 504 patients infected with HIV-1 (subtype B). From this cohort, 80 black/African American patients were identified and placed into the drugs-of-abuse subcohort using stringent definitions of drug abuse described in the Materials and Methods. The 80 patients were categorized into the following groups, 29 preferential nonusers (PN), 27 preferential cocaine users (PCo), 8 multidrug users (MDU), 11 preferential cannabinoid users (PCa) and 5 preferential benzodiazepine users (PBe). These 80 patients were used for the CCM analysis. An additional 23 black/African American patients were selected for the WLCM model, since they had varying levels of cocaine and had never tested positive for any other drug. Thus, the total number of black/African American patients in the drugs of abuse sub-cohort was 103 (Table 1).

Health of the Patients in the CCM analysis

To assess the impact of drug use on the overall health of the patients in the CCM model, a comparison of CD4+ and CD8+ T-cell counts, CD4:CD8 ratio, and viral load was performed. A normal $CD4^+$ T-cell count ranges from 500 to 1000 cells/μl, and a normal CD8+ T-cell count ranges from 300 to 1000 cells/μl.[25] While decreased levels of CD4+ T-cells and measurable viral loads are classical indicators of HIV disease severity, studies have shown that an elevated CD8+ T-cell count while a patient is on HAART, as is the case for the majority of the patients reported in the present study, can be a warning of treatment failure and is associated with accelerated HIV disease progression.[26] The PN population was healthier than either the PCo or MDU populations. The PN group exhibited a higher mean CD4+ T-cell count and lower mean CD8+ T-cell counts (Fig. 1). The CD4:CD8 ratios of the PCo and MDU populations were therefore lower than in the PN population (Fig. 1). The PCo and MDU populations also had higher mean viral loads than the PN population (Fig. 1).

Impact of Drugs of Abuse on Cytokine Profiles in Patients Infected with HIV-1

To understand the impact of drugs of abuse on cytokine profiles in patients within the drugs-of-abuse subcohort, plasma samples from the subjects were subjected to a cytokine human 30-plex immunoassay. The 30 cytokines were examined with respect to their association with cocaine use utilizing the two biostatistical models, CCM (n=80) and WLCM (n=103) (Fig. 2). CCM has been the model traditionally used for this type of analysis and in this regard it served as an informative control for comparison against the WLCM model, which was developed to allow for any measured drug (cocaine in this study) to have an effect on the value of each cytokine in the 30-plex panel. In order to understand the complicated nature of drug abuse, the WLCM analysis was performed in three different ways (AT-VISIT, UPTO-VISIT, and ALL-VISITS, as outlined above). This allowed us to define the effect of cocaine use over a long period on the levels of the 30 cytokines examined in the study, given the longitudinal sampling with the associated drug testing and detailed history of drug abuse available at every visit for every patient in the $D_{REXEL}M_{ED}$ HIV/AIDS Genetic Analysis Cohort. Since the WLCM model allows patients to not be grouped, it gave us the potential to add more patients into the analysis. In addition, the WLCM RESTRICTED AT-

VISIT analysis was performed with the same number of patients as in the CCM analysis to compare the results obtained from both models. Analysis using the CCM model identified epidermal growth factor (EGF) as being significantly associated with cocaine use (P=0.0203). Analysis using the same group of patients with the WLCM model (designated WLCM RESTRICTED AT-VISIT) also revealed EGF (P=0.0267) to be significantly associated with cocaine use as well as IL-4 (P=0.0134) and monokine induced by gamma interferon (MIG) (P=0.0480). Analysis using the WLCM model for the AT-VISIT identified eotaxin (P=0.0336) and IL-4 (P=0.0021) to be positively associated with cocaine use. trended positively associated as a group with cocaine use (P=0.0651). Analysis for the UPTO-VISIT identified IL-4 (P=0.0261) as being positively associated with cocaine use. In addition, in analyzing the effects of cocaine on the Th2 cytokine panel, the values of three cytokines, IL-4, IL-5, and IL-10, when added together were positively associated with cocaine use (P=0.0364). Finally, analysis for ALL-VISITS identified IL-10 (P=0.0208) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (P=0.0437) as being positively associated with cocaine use, as was the Th2 panel (P=0.0191) (Fig. 2 and Table 2).

In order to understand the impact of the results reported herein with respect to other clinical studies performed in HIV-1-infected individuals within the context of substance abuse, the literature was also reviewed in an attempt to identify previous studies performed on human plasma, serum, or blood or ex vivo studies of human PBMCs to determine cytokines/chemokines associated with HIV-1 and/or cocaine use/exposure (Fig. 2). Interestingly, to our knowledge, no studies on the scale reported herein have analyzed human samples for the combined effects of HIV-1 and cocaine, the closest being Kamat et al.[20] Of the studies performed concerning HIV-1 pathogenesis and disease in the absence or presence of cocaine, the statistical analyses utilized in prior studies ranged from relying on subjects' verbal admission of drug use to studies performed without the use of longitudinal sampling. In addition to the human studies, the single animal model study that has examined the effects of HIV and cocaine together, using a simian immunodeficiency virus macaque model, reported no effect of cocaine on the levels of IFN-β, MCP-1, IL-6, TNF-α, or IFN-γ.[27]

Cocaine Significantly Impacts Disease Severity in HIV-1-Infected Cocaine Users

Due to the longitudinal nature of the cohort and the novel WLCM model that has been developed and reported herein, it was possible to associate percentages of cocaine-positive HIV-1-infected clinical samples used with levels of cytokines that were shown previously to be impacted by cocaine use and clinical parameters of disease severity. These analyses are distinct from the clinical parameters shown in figure 2 due to the use of this novel model. This analysis was performed with patients who had tested positive for only cocaine and no other drug and patients who had not tested positive for any drug (n=64). This patient selection allowed analyzing the impact of pure cocaine use, which was varying in terms of percentages of cocaine-positive HIV-1-infected clinical samples, on the immunomodulatory profile. Percentages of cocaine-positive HIV-1-infected clinical samples were calculated by taking into account a patient's longitudinal drug use history as explained in Figure 3 legend. Panel A indicates that as the percentage of cocaine-positive HIV-1-infected clinical samples

increased, CD4+ T-cell counts decreased significantly and viral load increased significantly. With respect to the cytokines, panel B includes all the cytokines that were significantly associated with cocaine use using the WLCM AT-, UPTP-, or ALL-VISITS analyses, and showed IL-10 and GM-CSF as well as the Th2 panel of cytokines were all positively associated as the percentage of cocaine-positive HIV-1-infected clinical samples increased. Since this analysis was performed for all the 30 cytokines, panel C shows additional cytokines that were significantly associated with cocaine use such as EGF, IL-1β, IL-2, IL-7, VEGF, G-CSF as well as the Th1 to Th2 ratio (Fig. 3).

DISCUSSION

Using an exhaustive multiplex approach and a novel biostatistical model, we identified several cytokines that are associated with preferential cocaine use in the plasma of a longitudinal cohort of HIV-1-infected individuals who have detailed histories of drug abuse. Because the majority of the cohort was black/African American, data from only these individuals was presented. However, additional cytokine profiling data from a limited number of individuals of different races showed differential regulation of these cytokines based on race (data not shown). These studies also emphasize the detrimental impact of cocaine use on HIV-1 disease progression. Because of this link, the data was further analyzed with respect to individual cytokines and their association with viral load and/or CD4 T-cell counts. This analysis was performed with two methods. First, the same analysis was performed while considering cocaine as a confounder to VL and CD4 counts. Second, subcohorts of patients were analyzed that either exclusively use cocaine (the PC population) or never use cocaine (the PN population). In both cases, no cytokines or groups of cytokines were linked in the WLCM model after multiple testing correction (data not shown). This result is likely due to the fact that the patients selected for the analyses reported herein were patients that have been seen by the clinic three or more times. Consequently, their CD4 T-cell counts were shown to be consistently elevated and their viral loads were shown to be well controlled as a result of effective therapy (Fig. 1).. Consequently, the small level of variation in CD4 cell counts and/or viral load measurements cannot be accurately captured based on the small amount of variation in cytokine expression.

Studies have indicated that the phenomenon of cocaine regulating cytokine levels is mediated by corticosterone in spleen cells of cocaine-treated mice. In animals treated with cocaine, levels of IL-4 and IL-10 were increased but levels of IL-2 and IFN-γ were not changed, suggesting that while cocaine led to an increase in the Th2 response, it did so by not inhibiting the Th1 response, thus allowing the Th2 response to proceed unrestricted.[13] Another study, with chronically cocaine-treated rats, showed a rapid and sustained suppression of lymphocyte proliferation, which was supported by a decrease in the levels of IL-2, integral in the proliferation of lymphocytes and activated T lymphocytes. IL-10 also increased concomitantly, and the proinflammatory cytokine TNF-α and anti-inflammatory cytokines IL-10 and transforming growth factor-β1 were dysregulated. Transforming growth factor-β1 has been shown to shift the Th1/Th2 response towards Th2 cytokines. The investigators concluded that cocaine allowed for the dysregulation of cytokine expression.[14]

Thus, the identification of cytokines such as IL-4 and IL-10 that have been significantly altered by cocaine use in the current study is consistent with previous cohort studies of more limited size as well as animal studies.[28,29,30,31] Both cytokines are Th2-type cytokines secreted by Th2 cells. While IL-4 is involved in differentiation of naïve helper T cells (Th0) to Th2 cells, IL-10 is known to downregulate Th1 cytokines.[32,33,34,35,36,37] In fact, when comparing the results obtained with the Th1 and Th2 cytokine panels with clinical samples derived from HIV-1-infected patients in the DREXELMED cohort, there was a significant decrease in the ratio of Th1:Th2 cytokines that correlated with an increase in percentage of cocaine-positive HIV-1-infected clinical samples (Fig. 3). In conjunction with this information, cocaine has been implicated in dysregulation of the Th1/Th2 cytokine balance and in driving the immune response towards a Th2-type response.[13,38,39] Preferential Th1- and Th2-type responses account for several immunopathological disorders, such as allergic responses and fetal transplantation tolerance. During HIV-1 infection in particular, the imbalance between Th1/Th2 cytokines was significant, especially given that the bias towards a Th2-type response and an inhibition of Th1-type response has been associated with the immune system failing to control HIV-1 infection, leading to progression of the infection towards AIDS.[31,40,41] Clerici et al. have proposed that a switch from a Th1- to a Th2-type (specifically, IL-4 and IL-10) response could lead to increased intercellular susceptibility to HIV-1 as well as transmission,[40] possibly owing to the upregulation of co-receptors such as CXCR4 by cocaine exposure via signaling through the sigma-1 receptor.[42,43] This would favor the replication of CXCR4-utilizing HIV-1 strains or X4 viruses, specifically within Th2 cells that are able to secrete IL-4, which has also been shown to further upregulate CXCR4, whereas Th1 cytokines such as IFN-γ have been shown to downregulate CXCR4 expression.[44]

This study has also shown that clinical parameters such as CD4+ T-cell counts, and viral load correlate with percentage of cocaine-positive HIV-1-infected clinical samples, further supporting the conclusion that cocaine is detrimental to the immune system and facilitates the acceleration of HIV-1 disease progression. Building on the analysis of results performed in previously published studies, we have analyzed our data using a modern, robust biostatistical model that we developed in order to define the singular effect of cocaine on cytokine profiles. The presence of 30 cytokine expression levels suggests that some false positive findings are possible. This possibility has been minimized by further confirmation using dosage in the WLCM, and future validation studies in replication cohorts will help eliminate these false positives. The WLCM can be used in future studies to define the effects of other drugs on specific individual cytokines. Interestingly, while reviewing the results in the literature, we did encounter HIV-1-positive-only cohorts, which utilized plasma, serum, and PBMC supernatants and drugs-of-abuse (cocaine) cohorts, which utilized plasma and PBMC supernatants for cytokine profiling.[4,7,20,28–31,45–59] However, to our knowledge, there were no reports of cytokine profiles on HIV-1-positive cohorts that included detailed longitudinal histories of cocaine use by members of a given cohort, as demonstrated here with the DREXELMED HIV/AIDS Genetic Analysis Cohort.

Acknowledgments

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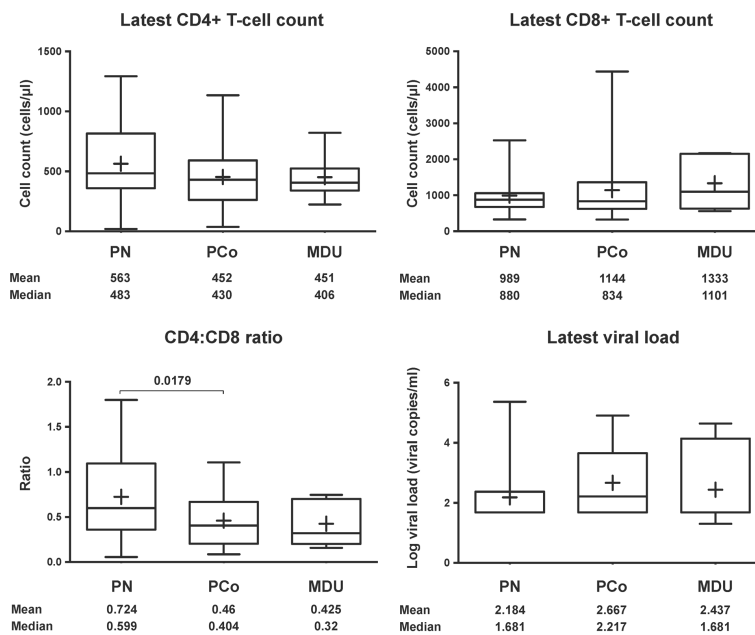
Latest CD4+ T-cell count

	PN	PCo	MDU
Mean	563	452	451
Median	483	430	406

Latest CD8+ T-cell count

	PN	PCo	MDU
Mean	989	1144	1333
Median	880	834	1101

CD4:CD8 ratio

0.0179

	PN	PCo	MDU
Mean	0.724	0.46	0.425
Median	0.599	0.404	0.32

Latest viral load

	PN	PCo	MDU
Mean	2.184	2.667	2.437
Median	1.681	2.217	1.681

FIGURE 1.

Drug use increased HIV-1 clinical severity in the DREXELMED Cohort. The latest CD4+ and CD8+ T-cell counts and the latest viral loads in the PN, PCo, and MDU groups are shown, which reflected the range and health of the cohort. The mean (+) and median values for each group are provided. A standard Student's t-test was performed. The latest viral loads were $\log_{10}$ transformed for generating plots.
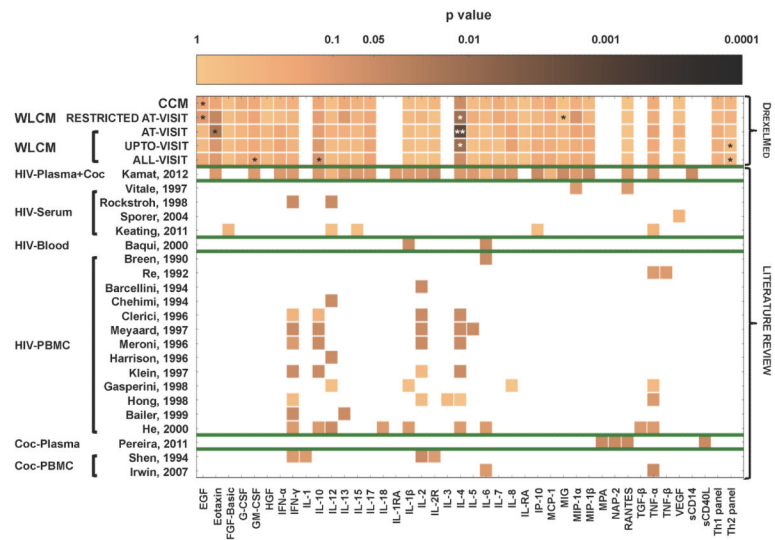
FIGURE 2.

Cytokines are significantly associated with cocaine use. In the studies on samples from the DREXELMED cohort, cytokines were analyzed using the categorical contribution model (CCM) (n=80) and three variations of the weighted linear contribution model (WLCM) (n=103) as well as RESTRICTED AT-VISIT using WLCM (n=80), adjusting for age, gender, highly active antiretroviral therapy (HAART) status, and hepatitis C virus (HCV) coinfection as confounders, were expressed as a heat map with respect to their p-values. The p values were denoted in log scale and as the color gets darker, the p value becomes more significant. Values of $p < 0.05$ were considered statistically significant and marked with an asterisk. Those marked with a double asterisk were also significant after multiple test comparison using the Benjamini-Hochberg correction; q 0.05. The effect size and p value are shown in Table 2. Cells that lack color were not measured in that particular study. The Th1 panel consists of interferon-γ (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α), while the Th2 panel consists of IL-4, IL-5, and IL-10. AT-VISIT results take into consideration drug use at a particular visit only. UPTO-VISIT results take into consideration drug use up to that particular longitudinal visit, and ALL-VISIT results take into consideration drug use at all of the patient's longitudinal visits. Cytokine profiles from the drug abuse subcohort were also compared with other published cohort reports. The molecules studied and the p values determined (regardless of biostatistical method) in these reports are expressed in the heat map with only those that were significant indicated (therefore no color in these analyses equates to not analyzed or not significant). The green lines in the figure are used to segregate cytokine results from different sample types such as serum, blood, and so on. EGF, epidermal growth factor; FGF, fibroblast growth factor, HGF, human growth factor; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MPA, medroxprogesterone acetate; NAP-2, neutrophil-activating protein-2; RANTES, regulated on activation, normal T-cell expressed and secreted; TGF-β, transforming growth factor-β; and VEGF, vascular endothelial growth factor.
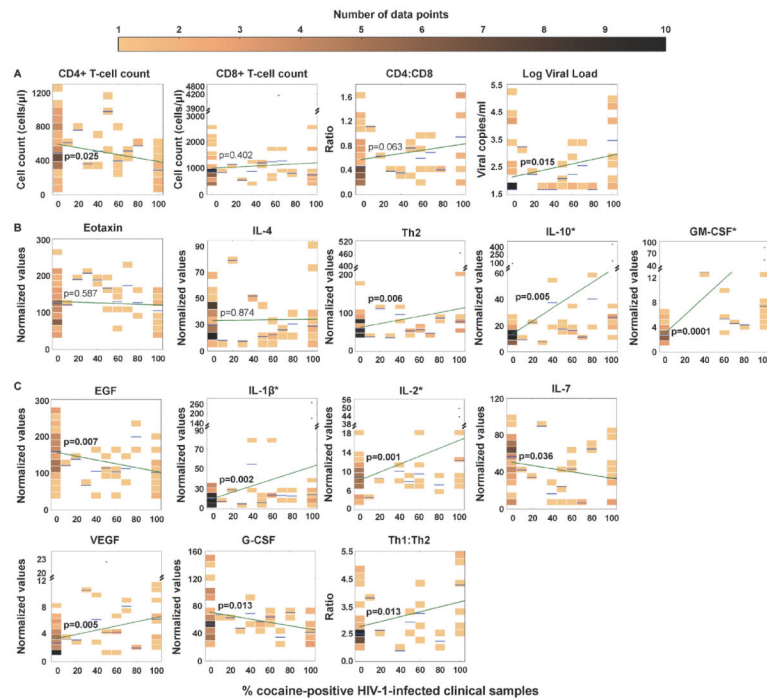
FIGURE 3.

Increase in percentage of cocaine-positive HIV-1-infected clinical samples leads to increase in HIV-1 disease severity. 2D histograms were graphed with respect to percentage of cocaine-positive HIV-1-infected clinical samples across longitudinal samples for an n of 64 patients, which included patients in the preferential nonuser (PN) and preferential cocaine user (PCo) groups as well as other only cocaine users who had varying levels of cocaine and had never tested positive for any other drug. The histograms at 0% in all graphs correspond to the value of the cytokine of the sample tested or the clinical measurement associated with the same sample of patients in the PN group. The percentage of cocaine-positive HIV-1-infected clinical samples was calculated as the fraction of longitudinal visits with a positive cocaine test for patients in the PCo group. For example, someone with four longitudinal samples in which two cocaine tests were positive and two were negative would be represented at 50%. Some histograms are missing between selected cytokines for patients at a given percent of cocaine-positive HIV-1-infected clinical samples because the cytokine measured was out of the linear range (i.e., compare GM-CSF and eotaxin). Cytokine values have been represented as the normalized and adjusted values used for the biostatistical analysis. (A) Analysis in relation to HIV clinical parameters. The cytokines shown here are all the cytokines that were shown to be significantly associated with cocaine in Figure 2 using the WLCM AT, UPTO, and ALL-VISITS analyses (B). Additionally, six cytokines, EGF, IL-1β, IL-2, IL-7, VEGF, and G-CSF, as well as the Th1:Th2 ratio were found to have a significant p-value, when analyzing this set of patients (C). The p-values indicated represent the likelihood of finding a correlation coefficient of the observed magnitude or better by chance and the significant p-values have been shown in bold. Those marked with an asterisk were significant when corrected for multiple testing (Benjamini-Hochberg). The number of data points in each histogram is indicated in a color scale panel. As the color gets darker, the number of data points increases. The green lines in each of the figures represents

the trend line, and the blue bar represents the median values. EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; and VEGF, vascular endothelial growth factor.

TABLE 1

Demographics of the DREXELMED HIV/AIDS Genetic Analysis Drugs-of-Abuse Subcohort containing 103 black/ African American individuals

Demographic Variables	Categories	Count (%)/Mean (± SD) with Clinical Variables (n=103)
Gender	Male	60 (58.3%)
	Female	43 (41.7%)
Drug Use	Tobacco	61 (59.2%)
	Alcohol	45 (43.7%)
	Amphetamines	0
	Barbiturates	0
	Benzodiazepines	7 (6.8%)
	Cannabinoids	18 (17.5%)
	Cocaine + metabolite	44 (42.7%)
	Opiates	0
	Phenylcyclidine	0
HAART status	cH	87 (84.5%)
	dH	5 (5.8%)
	nH	11 (10.7%)
Age		47 (± 9.5)
Years since diagnosis		11.6 (±6.759)
HCV status	Positive	44 (42.7%)
	Negative	57 (55.3%)
	ND	2 (1.9%)
CD4		517 (± 288)
CD8		1097 (± 706)
Log Viral Load		2.48 (±1.09)

cH, continuous HAART (highly active antiretroviral therapy), dH, discontinuous HAART; HCV, hepatitis C virus; nH, naïve HAART; ND, not determined

TABLE 2

Effects for all cytokines significantly associated with cocaine use determined by the WLCM biostatistical model

Cytokine	Effect (p value)		
	AT-VISIT	**UPTO-VISIT**	**ALL VISIT**
Eotaxin	25.93 (0.0336)	—	—
GM-CSF	—	—	8.74 (0.0437)
IL-10	—	—	29.07 (0.0208)
IL-4	14.25 (0.0021)*	11.19 (0.0261)	—
Th2 panel	—	27.99 (0.0364)	32.83 (0.0266)

The effect signifies the effect cocaine has on the value of each cytokine. A minus sign in front of the effect value denotes that the drug has a negative effect on the value of the cytokine (downregulation), while no sign in front of the effect value denotes that the drug has a positive effect on the value of the cytokine (upregulation). A asterisk signifies the correlation was significant when corrected for multiple testing (Benjamini-Hochberg). GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; WLCM, weighted linear contribution model.