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Cellular immunology in HIV-1 positive african american women using alcohol and cocaine

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1. ABSTRACT

Co-use of illicit drugs, in particular cocaine and alcohol, is common among HIV-1+ men and women of different ethnic groups. We compared cohorts of alcohol and cocaine co-users HIV-1+ African American women and in cohorts of drug-free, or methamphetamine users HIV-1+ men. We monitored clinical cellular immune parameters at repeated regular intervals. We found that significant inverse correlations between CD8+CD38+ cells and subpopulations of CD4+ cells distinguished by the expression of CD45RA in HIV-1+ alcohol and cocaine co-users but not in drug-free HIV-1+ patients. Following stratification for CD4+ cell number, we found the CD4+CD45RA+ subpopulation to be significantly higher ($p<0.05$) in the drug user compared to drug-free HIV-1+. Drug abuse may alter the change from the CD4+CD45RA+ to the CD4+CD45RA- phenotype selectively, which recovers in HIV-1+ methamphetamine abusers during treatment from baseline to 4-weeks, as manifested by improved IL-2 production *in vitro*. of TH1 and TH2 cytokines during progression to AIDS.

2. INTRODUCTION

More than 10 million individuals abuse illicit drugs in the U.S. Intravenous drug abusers make up the population at second highest risk for infection with human immunodeficiency virus-1 (HIV-1). Cocaine is a highly abused drug in Miami (1,2). In addition, cocaine abuse is associated with acceleration of HIV-assocatied dementia (3). The trafficking and abuse of methamphetamine is alarmingly high and increasing in the Pacific Southwest, and Los Angeles is one of the primary markets of distribution for this drug.

Joint abuse of illicit drug, in particular cocaine and alcohol, is common among HIV-1-seropositive men and women in all ethnic groups. groups. Cocaine stimulates HIV-1 replication in cell culture *in vitro* (4,5). In the drug abuser, liver microsomal carboxylesterases transesterify cocaine in the presence of ethanol to produce cocaethylene (6,7). In addition to cocaine stimulation of HIV-1 replication in cocaethylene does so as well (8-10). Serious concerns were raised about the immunotoxicity of alcohol, cocaine, and cocaethylene. Alcohol, cocaine, and cocaethylene have been proposed as putative co-factors of the immunopathogenesis that is associated with the progression to the acquired immune deficiency syndrome (AIDS) (7-12, 19-21). Cocaine stimulated HIV-1 replication in a mouse model (13). In an additional mouse model of joint alcohol and cocaine abuse, anabolism of cocaethylene following administration of alcohol was demonstrated to enhance the immunotoxicity of cocaine. Our data in cohorts of drug abusers in Miami and Los Angeles support the hypothesis that joint alcohol and cocaine abuse exacerbates HIV-1-related immunopathology and indicates that cocaine alone, alcohol alone, cocaine and alcohol mixtures, and cocaethylene blunt functional and maturational responses of CD4+ T cells in these subjects (14-18).

The identification and characterization of factors that yield predictive information about the rate of progression of HIV-1 seropositive individuals to AIDS is important because the time course of progression is critical for the success of immunotherapeutic intervention modalities (22). Immunopathological markers of disease progression, such as the number of peripheral blood CD4+ T lymphocytes, are commonly used (22-24,31). The proportion of AIDS clinical diagnoses increases as the number and percent of circulating CD4+ T cells decrease. Many patients with CD4 counts between 300-500 may have clinical signs of immune suppression. As the CD4 count drops (200-300 cells), the proportion of patients with signs of immune suppression increases until they reach levels below 200, which signifies the AIDS diagnosis.

The rate of progression to AIDS can now be better characterized by the identification of certain subpopulations of CD4+ or CD8+ T cells defined by the expression of certain markers (e.g., CD45RA, CD62L or CD38) (25-28,30), by the assessment of soluble products of immune activation (e.g., neopterin and beta2-microglobulin) (29), as well as by the determination of the circulating levels of certain cytokines (e.g., TH1 vs. TH2) (32-36), and other functions of T cells (37,38). These laboratory markers of disease progression have been established using populations of HIV-1-seropositive Caucasian men, and there remains a dearth of information about the clinical immunopathological profile of HIV-1-seropositive women in general and of patients in specific ethnic groups.

In this prospective mixed cohort study, we have monitored clinical parameters of immunopathological progression to AIDS in HIV-1-seropositive and HIV-1-seronegative African-American women of child-bearing age, the majority of whom have a history of joint alcohol and cocaine abuse. We have characterized this sample at regular repeated 6-month intervals. We have obtained parallel measurements in a group of drug-free HIV-1-seropositive Caucasian men, in methamphetamine HIV-1-seropositive men, and in drug-free HIV-1-seronegative men and women for reference. We show that the two sample populations of HIV-1-seropositive patients do not differ significantly when the standard clinical immunological parameters of HIV disease are used. We also compare African-American drug using HIV-1-seropositive women, and show that they have significantly more circulating CD4+CD45RA+ lymphocytes compared to Caucasian drug-free HIV-1-seropositive men matched for CD4+ T cell number between 300 and 200 cells per cc. Further, we compare the percent peripheral blood CD4+ lymphocytes in the CD45RA- and in the CD45RA+ compartments of methamphetamine HIV-1 seropositive men recovered significantly during the period of treatment from baseline to 4-weeks, and establish that this recovery of cellular immunity integrity also corresponded with improved whole blood production of IL-2 in response to mitogenic stimulation *in vitro*. The importance of these observations for the development of new and improved immunotherapeutic modalities for HIV-1-seropositive multiple drug abusers is discussed.

3. MATERIALS AND METHODS

3.1. Subjects and Design

The data reported here were obtained from sample populations that consist of subjects recruited in two independent studies in Miami and Los Angeles risk locales and catchment areas. HIV-1-seropositive African-American women (n=63) were recruited as part of the Miami project. HIV-1-seronegative African-American women were also recruited in Miami as the drug-use control group (n=41). A large proportion of these subjects were followed at 6-month intervals, as indicated in Table 1.

For comparison purposes, we recruited in Los Angeles, a group of drug-free HIV-1-seropositive Caucasian men matched for age and socio-economic level (n= 10). An additional component of the Los Angeles group was a control that consisted of HIV-seropositive methamphetamine drug abusers, followed for up to 4 weeks into behavioral treatment for methamphetamine

Table 1. Units of Measurement for Each Sample Group

Subject Group	1	2	3	4	5	TOTAL
HIV-1 Seropositive						
Drug User	21	26	7	8	1	131
Drug-Free	4	3	3	0	0	19
HIV-1 Seonegative						
Drug User	31	10	0	0	0	51
Drug Free	15	0	0	0	0	15

abuse. Methamphetamine dependence was established by DSM-IV criteria. Subjects (mean age 35.0±6.3 years), from Asian (n=1) or Caucasians (n=3) ethnic backgrounds, were long-time methamphetamine users (8.1 ± 7.0 years) by means of injection (n=3) or insufflation (n=1). Whereas the subjects were diagnosed as HIV-seropositive, none received antiretroviral medications during the study period. A third control group consisted of HIV-1-seronegative, healthy, drug-free, normal control men and women from the principal ethnic groups in the U.S. recruited in Los Angeles. These subjects constituted the normal control drug-free reference group (n= 15). All patients and normal subjects signed Informed Consent approved by the respective institutions and reviewed and renewed annually. Patients were followed at repeated semi-annual intervals for a period of up to four years. Normal subjects were tested on one visit to the study.

3.2. Samples

Peripheral blood samples were collected from an antecubital vein with EDTA as anticoagulant. Baseline measurements consisted of duplicate collections obtained 48 hours apart to establish intra-donor variability. Follow-up measurements consisted of one collection only. When possible, samples were collected between 15:00 and 17:00 hours, with participants instructed to take no food, caffeine, or nicotine for at least 1 hour prior to sample collection to minimize neuroendocrine-mediated variability in immune measurements. When applicable, ten-minute saliva samples (2.0-5.0 ml) were collected, clarified by centrifugation (2,000 rpm, 20 min). Samples were kept at room temperature with gently shaking for up to 48 h while in shipment. Samples were centrifuged (1,500 rpm, room temp, 10 min). Plasma aliquots were cryopreserved at -70 °C for batch neopterin, beta2-microglobulin and cortisol assays. Buffy coats were used for all immunophenotypic assessments following determination of white blood cell count (WBC, expressed as cell number x 10^6 per cc) and lymphocyte differential (expressed as % of WBC) with a clinical Coulter counter (18,21).

3.3. Immunophenotyping

Lymphocyte subpopulations were monitored by standard whole blood flow cytometry. In brief, 50 microliters of buffy coat were incubated with 0.01 ml of fluorochrome-conjugated mouse anti-human monoclonal antibody diluted with 0.04 ml of phosphate buffered saline (PBS) supplemented with 5 fetal calf serum (GIBCO) (5F-PBS) (room temp, 30 min). The antibodies used were fluorescein (FITC) conjugated anti-CD45RA, phycoerythrin (PE)-conjugated anti-CD4, and FITC-conjugated anti-CD8 and PE-conjugated anti-CD38 (Becton Dickinson; Mountain View, CA). Stained cells were washed twice with excess 5F-PBS, depleted of red blood cells by osmotic shock lysis, and paraformaldehyde fixed before flow cytometric analysis with a flow cytometer instrument (FACScan, Becton Dickinson). The percent data produced by the flow analysis were transformed to absolute numbers using WBC and differential (18,21,23,29).

3.4. Plasma Markers & IL-2 Production

To assess plasma cortisol, neopterin and beta2-microglobulin levels, plasma aliquots were batch-tested with commercially available radioimmunoassay kits (cortisol and neopterin: ICN Biochemicals; beta2-microglobulin: Pharmacia), as per the recommendations of the manufacturers. Cortisol was tested within the range of 1 microgram/dl-100 microgram/dl. Sensitivity was assessed with a minimum detectable dose of 0.15 microgram/dl. The specificity of the assay is greater than 95%, and the inter-assay coefficient of variation is 6.0%-7.9%. Neopterin was tested within the range of 0.5 ng/ml-100 ng/ml. Sensitivity was assessed with a minimum detectable dose of 0.05 ng/ml. The specificity of the assay is greater than 95%, with a coefficient of variation of 7.3%-12.7%. beta2-microglobulm was tested within the range of 0.2 mg/L-32 mg/L. Sensitivity was assessed with a minimum detectable dose of 0.15 mg/L. The specificity of the assay is greater than 95%, and the coefficient of variation is 4.4%-5.2%. Plasma and saliva were tested for IL-2 and IL-4 levels using commercially available ELISA kits (Biosource; Camarillo, CA) (sensitivity: 8.7 pg/ml, linearity: 15.6-1000 pg/ml, intra-assay and inter-assay reproducibility of 5% and 7%).

Whole blood IL-2 production was obtained by stimulating whole blood. In brief, buffy coats were obtained, washed twice by centrifugation in RPMI, reconstituted with equal volume of 10% human serum-RPMI, diluted (1:4) in the same culture medium, and stimulated with PHA (5 microgram/ml, 72 h). At harvest, cells were centrifuged, and conditioned filtered to remove cell debris, and stored at -70^o C until batch assay for IL-2 as described above (24,38).

3.5. Data Analysis

Data are presented as mean±standard deviation (mean $\pm$ SD) unless otherwise indicated. Correlation coefficients were computed as the Pearson product-moment correlation. Data were analyzed by means of non-parametric statistics (Wilcoxon Rank Sum test) because of the unevenness of the group distributions due to substantial individual differences. The samples were stratified based upon CD4+ T lymphocyte number. Significance level was set at alpha= 0.05.

4. RESULTS AND DISCUSSION

4.1. Cellular Immune Parameters

Table 2 presents the clinical cellular immune parameters we have examined in this study. We have compared drug using HIV-1-seropositive African-American women (total number of units of measurement:

Table 2. Immune Parameters Among HIV-1+ and HIV-1- Drug-Free and Drug-Abusers

	HIV-1 Seropositive		HIV-1 Seronegative	
Cellular Immune Parameter	**Drug Abusers**	**Drug Free**	**Drug Abusers**	**Drug Free**
Mean	n = 131	n = 19	n = 51	n = 15
CD4 [1]	0.52±0.33	0.37±0.10	1.06±0.61	0.86±0.41
CD4% [2]	26.37±12.93	21.63±5.49	41.00±15.15	38.60±10.15
CD8[3]	0.99±0.53	0.89±0.34	0.90±0.39	0.74±0.29
CD8%	50.24±14.77	51.00±13.76	38.41±16.41	35.07±10.98
RATIO	0.63±0.47	0.47±0.22	1.37±0.91	1.37±1.04
CD4 + CD45RA+[3]	0.19±0.16	0.11±0.07	0.36±0.26	0.42±0.25
CD4 + CD45RA+%	9.62±6.11	6.42±4.02	13.76±7.61	18.93±7.07
CD4+CD45RA-[3]	0.41±0.29	0.26±0.10	0.81±0.44	0.44±0.22
CD4+CD45RA-%	20.47±10.82	15.11±4.43	32.04±12.65	19.93±6.70
RATIO	0.57±0.47	0.48±0.39	0.55±0.36	1.06±0.49
CD8+CD38+[3]	0.54±0.42	0.62±0.29	0.35±0.25	0.29±0.10
CD8+CD38+%	27.24±14.03	36.21±14.63	15.45±13.14	15.22±6.57
CD8+CD38-[3]	0.29±0.22	0.26±0.18	0.35±0.23	0.22±0.15
CD8+CD38-%	14.73±8.93	14.79±7.57	14.14±7.07	10.78±6.96
RATIO	2.75±2.18	3.25±2.42	1.37±1.16	2.05±1.88

[1]: Cell number ([3]) is expressed as mean x 10^6 per cc ± SD, [2]: Cell percent (%) is expressed as mean % gated lymphocyte region ± SD

131), and drug using HlV-1-seronegative African-American women (total number of units of measurement: 51), with drug-free HIV-1-seropositive Caucasian men (total number of units of measurement: 19), and drug-free HlV-1-seronegative normal healthy control men and women from the principal ethnic groups in the U.S. (total number of units of measurement: 15) as controls.

The data given in the table indicate that the two groups of HlV-1-seronegative subjects did not differ significantly in terms of the percent and absolute number of CD4+ and CD8+ cells, the CD4/CD8 ratio, or the percent or absolute number of circulating subpopulations of CD8+CD38+ cells . The data in the Table also show that the CD8+CD38+/CD8+CD38- ratio was also not different between the groups of drug user and drug-free HlV-1-seronegative subjects. It is noteworthy to observe that drug-free HlV-1-seronegative subjects appeared to have almost half the percent and number of CD4+CD45RA- cells compared to HlV-1-seronegative drug abusers. The ratio of CD4+CD45RA+/CD4+CD45RA- cells (mean ± SD) 1.06±0.49 in the drug-free HlV-1-seronegative subjects, was close to twice that observed in the HIV-1-seronegative subjects who jointly abuse alcohol and cocaine (Table 2). These differences were not statistically significant.

The data in Table 2 also indicate that the two groups of HIV-1-seropositive patients were comparable in terms of the clinical cellular immune parameters we have examined. The percent and absolute number of CD4+ and CD8+ cells were comparable in both groups, and the CD4/CD8 ratio did not differ significantly between the sample of drug using HIV-1-seropositive African-American women compared to drug-free HIV-1-seropositive Caucasian patients. We also recorded no statistical differences in the percent or absolute number of circulating subpopulations of CD8+CD38+ cells or in the CD8+CD38+/CD8+CD38- ratio between these two groups. The observation that drug-free subjects may have fewer circulating CD4+CD45RA- cells compared to drug abusers, which we noted above was reiterated among the HIV-1-seropositive patients. This difference and the difference in the CD4+CD45RA+/CD4+CD45RA- ratio were not statistically significant (Table 2).

The data in Table 2 further show that drug user HIV-1-seropositive patients had fewer CD4+ cells, and therefore a lower CD4/CD8 ratio compared to drug user HIV-1-seronegative subjects. HIV-1-seropositive patients also have fewer CD45RA+ and CD45RA CD4+ cells compared to drug user HIV-1-seronegative subjects, although the ratio of these two CD4+ cell subpopulations (CD4+CD45RA+/CD4+CD45RA-) remained unaltered. HIV-1-seropositive drug user patients had about twice the percent of activated CD8+ cells compared to HIV-1-seronegative drug user subjects, and this difference was reflected as well in the CD8+CD38+/CD8+CD38- ratio between the two groups (Table 2: 1.37±1.16).

Table 3 presents the values of the clinical cellular immune parameters under study in a subgroup of drug user (total number of units of measurement: 21) and drug-free (total number of units of measurement: 7) HIV-1-seropositive patient samples stratified for CD4 + cell number between 200 and 300 per cc, because this range represents a critical stage of progression to AIDS. The data in the table indicate that these two subgroups of HIV-1-seropositive patients did not differ significantly in the percent and absolute number of CD4+ cells. The data in the table also show that these two subgroups were comparable for the percent and number of circulating CD8+ cells, as well as for the CD4/CD8 ratio. These two subgroups of HIV-1- seropositive patients could also not be distinguished by the percent and absolute number of circulating subpopulations of CD8+CD38+ cells and in terms of the ratio CD8+CD38+/CD8+CD38-.

The percent and number of circulating CD4+CD45RA- cells were also not statistically different in these two subgroups of HIV-1-seropositive patients. By contrast, the percent and the number of CD4+CD45RA+ cells were significantly higher ($p < 0.05$) in the group of HIV-1-seropositive patients who jointly abused alcohol and cocaine compared to drug-free HIV-1-seropositive

Table 3. Comparative Immunopathology Among Drug-Abuser and Drug-Free HIV-1 Seropositive Patients

	HIV-1 Seropositive (200-300 CD4+ Cell Number)	
Cellular Immune Parameter	**Drug Abusers**	**Drug Free**
Mean	n = 21	n = 7
CD4 [1,3]	0.24±0.02	0.26±0.03
CD4% [2]	14.95±5.63	15.57±2.76
CD8 [3]	1.15±0.65	1.03±0.29
CD8%	58.19±14.98	61.00±12.01
RATIO	0.30±0.19	0.26±0.07
CD4 + CD45RA+[3]	0.10±0.07	0.05±0.03
CD4 + CD45RA+%	5.81±4.57	2.86±1.57
CD4+CD45RA-[3]	0.24±0.17	0.21±0.05
CD4+CD45RA-%	13.62±6.27	12.71±3.55
RATIO	0.52±0.47	0.27±0.20
CD8+CD38+[3]	0.68±0.54	0.81±0.28
CD8+CD38+%	33.67±19.31	48.00±13.00
CD8+CD38-[3]	0.19±0.17	0.22±0.15
CD8+CD38-%	10.05±7.70	13.00±8.74
RATIO	5.68±5.25	5.12±2.86

[2]: Cell number ([3]) is expressed as mean x 10^6 per cc ± SD, [2]: Cell percent (%) is expressed as mean % gated lymphocyte region ± SD

patients matched for CD4+ cell number between 200 and 300 per cc. This difference was reflected in the CD4+CD45RA+/CD4+CD45RA- ratio (Table 3). The rise in the percent of circulating CD8+CD38+ cells was significantly inversely correlated with the drop in the percent circulating CD4+ cells ($r=-0.51$, $p<0.05$) and with the drop in CD4+CD45RA- cells ($r=-0.48$, $p<0.05$) in the subgroup of drug user HIV-1-seropositive patients with CD4+ number between 200 and 300 per cc. Similar statistically significant inverse relationships were obtained in a subgroup of drug user HIV-1-seropositive patients with CD4+ number between 300 and 500 per cc (CD8+CD38+ vs. CD4+: $r=-0.42$, $p<0.05$; CD8+CD38+ vs. CD4+CD45RA-: $r=-0.36$, $p<0.05$). These associations were not observed in the group drug-free HIV-1-seropositive patients matched for CD4+ cell number.

In the control group of HIV-1-seropositive methamphetamine abusers, we observed a consistent recovery in the percent peripheral blood CD4+ lymphocytes in the CD45RA- and in the CD45RA+ compartments during the period of treatment. This recovery, which corresponded with methamphetamine abstinence/reduction in use between baseline and 4-weeks, paralleled the reported reduction of drug and sexual risk behaviors that might expose individuals to pathogens. This recovery in CD4+ cells also corresponded with improved whole blood production of IL-2 in response to mitogenic stimulation *in vitro*.

4.2. Soluble Immune Parameters

Not shown in Table 2 are the assessments of soluble markers of immune activation, neopterin and beta2-microglobulin, for the two groups of HIV-1-seropositive patients. We found no differences in plasma neopterin concentration (ng/ml mean $\pm$ SD; drug users: 5.78 ± 4.70, drug-free: 5.85 ± 2.86), or in plasma beta2-microglobulin concentration (mg/L mean ± SD; drug users: 3.02 ± 2.31, drug-free: 2.06 ± 0.69). Plasma cortisol concentrations also were not different between the two groups of HIV-1-seropositive patients (microgram/dl mean $\pm$ SD; drug users: 26.01 ± 11.94, drug-free: 27.26 ± 10.91). The rise in the percent of circulating CD8+CD38+ was significantly inversely correlated with the drop in the percent circulating CD4+ cells ($r=-0.46$, $p<0.05$) as well as with the drop in the percent circulating CD4+CD45RA- cells ($r=-0.53$, $p<0.05$) in drug user HIV-1-seropositive patients. By contrast, a significant inverse correlation coefficient was obtained only between the percent of circulating CD8+CD38+ cells and the percent of circulating CD4+ cells ($r = -0.56$, $p < 0.05$), but not the percent of circulating CD4+CD45RA- cells in the group of drug-free HIV-1-seropositive patients.

In the control group of HIV-1-seropositive methamphetamine abusers, salivary and plasma levels of IL-2 showed a significant positive correlation ($r=0.88$, $p<0.05$, $df=7$) across the participants and the repeated assessments. Similarly, the values for the IL-2/IL-4 ratio obtained in saliva and plasma across the participants at the different time points were strongly, albeit not significantly (alpha=0.05) correlated ($r=0.65$, $p<0.10$, $df=7$). Confirming the fact that salivary cytokine assessments were not contaminated by plasma or serum carry-over due to soft or hard tissue lesions or crevicular exudates, our data established that salivary and plasma levels of IL-4 were not correlated ($r=0.05$).

4.3. Fundamental Implications

Animal and *in vitro* studies converge in an emerging body of literature that suggests an important role for alcohol, cocaine and their metabolite, cocaethylene, in the immunopathogenesis associated with the progression to AIDS, as well as the replication cycle of HIV-1 (11-14,19-21,39-41). This study was designed to begin to establish whether or not these observations are relevant to clinical populations of HIV-1-seropositive patients who jointly abuse alcohol and cocaine. For this purpose, we examined clinical cellular immune parameters in a group of drug user HIV-1-seropositive African American women at repeated regular 6-month intervals for a period of up to 40 months. We obtained parallel assessments from HIV-1-seropositive drug-free patients as well as from HIV-1-seronegative joint alcohol and cocaine abusers and drug-free control subjects. We established that the two groups of HIV-1-seropositive patients under study could not be distinguished based upon the traditional clinical immunological parameters, including

the percent or absolute number of circulating CD4+ and CD8+ lymphocytes, CD4/CD8 ratio, plasma levels of neopterin or beta2-microglobulin, and of plasma cortisol levels. We also found that HIV-1-seropositive drug user and drug-free patients were indistinguishable from the perspective of certain subpopulations within the CD4+ and the CD8+ subsets (i.e., CD4+CD45RA+, CD4+CD45RA-, CD8+CD38+), which are significant markers of the immunopathology associated with the progression to AIDS (23,27-29).

In related characterizations of our sample populations, we established that urine cocaine and cocaethylene levels were significantly correlated in the HIV-1-seropositive patients as well as in the HIV-1-seronegative drug use subjects. Plasma neopterin levels were also significantly correlated with the peak of viral p24 expression by cultured monocytes in the HIV-1-seropositive drug abusing patients. By contrast, the production of IL-2 in response of whole blood to stimulation with the T cell mitogen phytohemagglutinin (PHA) was vigorous in the HIV-1-seropositive drug user patients, albeit blunted compared to that obtained from HIV-1-seronegative subjects (data not shown).

Taken together, these data appear to support the emerging literature, which indicates that there may not be a significant role of alcohol and cocaine in precipitating the progression to AIDS (39-41). Alcohol may primarily impair cellular immune events, thus decreasing immune competence (42). However, when we stratified our HIV-1-seropositive patients groups for CD4+ cell number, and compared drug user and drug-free patients with CD4+ cell number between 300 and 200 per cc, we found that the percent and number of CD4+CD45RA+ cells was significantly higher in the drug user compared to the drug-free HIV-1-seropositive patients. The relationship between the rise in CD8+CD38+ cells and the CD45RA+ and CD45RA- CD4+ subpopulations, a measurement whose relevance in the context of the progression to AIDS among HIV-1-seropositive drug user was recently emphasized, confirmed that fine immunopathological differences may distinguish drug user HIV-1-seropositive patients from drug-free patients at the critical stage of progression to AIDS characterized by CD4+ cell number in the range of CD4 cell number of 300-200 per cc.

The observations we have made on the control group of HIV-1-seropositive methamphetamine abusers further confirm the literature that TH1 and TH2 cytokines can be assessed reliably in saliva of HIV-seropositive patients (43). Our data further indicate that salivary assessment of IL-2 and IL-2/IL-4 ratio, but not IL-4 *per se*, approximate well serum measurements. The rise in salivary and plasma IL-2 concentrations we have observed during methamphetamine treatment confirms the literature, which shows that amphetamine causes significant suppression of IL-2 production *in vitro* (44), and suggests that these effects are reversible even in long-term abusers. Our data, which suggest that treatment for methamphetamine abuse has little effect on blood IL-4 levels, also confirm that methamphetamine does not alter IL-4 production *in vitro* (44). The correlation in IL-2/IL-4 ratio in saliva and plasma suggests that this important immune outcome measure can be easily obtained by testing saliva samples. That these observations hold across a small sample of HIV-infected individuals and across time strengthens the argument for using salivary measures, as it demonstrates the sensitivity of saliva to detect and to account for changes in plasma IL-2 and the IL-2/IL-4 ratio known to occur during progression of HIV disease (43).

4.4. Clinical Implications

The observations reported here are important because of the differential role of naive and memory CD4+ cells in the generation of TH1 and TH2 cytokines (21,32-34,42). These patterns of cytokines have been reported to represent a critical parameter in the immunopathology of the progression to AIDS, although there remains a number of controversial issues in the literature in this context (34,35). To begin to address this issue directly, we have gathered preliminary observations of a dramatic blunting in the expression of the CD7 marker following stimulation (diluted whole blood stimulated with PHA as above). CD7 is a marker reported to be associated with the maturation process of CD4+ T lymphocytes (36). Drug user and drug-free HIV-1-seronegative subjects show a 24.9±8.3 increase in the CD4+ CD7 + population following stimulation of whole blood as described; and a representative drug-free HlV-1-seropositive patient show a 16 rise in the CD4+CD7 + population following stimulation. By contrast, a representative drug user HlV-1-seropositive patient, matched for CD4+ cell number, only showed a 4.1 change in the CD4 + CD7 + population following stimulation (45). Taken together, our evidence suggests that joint alcohol and cocaine abuse may exacerbate the selective imbalance in functional and phenotypic characteristics of naive and memory T cells previously described in drug-free HlV-1-seropositive patients during progression to AIDS (37).

Abuse of illicit analogs of methamphetamine (i.e., Ecstasy, and other 'designer drugs') represents a growing social and public health problem. Individuals who use these drugs engage in high-risk behaviors for HIV and exhibit impaired immune host defenses that recover relatively quickly upon drug discontinuation. The observations noted here are timely and important because they establish that collection of whole saliva suffices to obtain reliable information about immune improvements among HIV-seropositive methamphetamine abusers undergoing behavioral substance abuse treatment.

5. CONCLUSIONS

In conclusion, our data suggest that joint abuse of alcohol and cocaine may alter the transformation of CD45RA+CD4+ cells to CD45RA-CD4+ cells. This outcome may not be evident in standard clinical immune assessments since, as we and others have observed, drug user and drug-free HlV-1-seropositive patients are otherwise indistinguishable. Our findings may be relevant to the development of immunotherapeutic modalities, including gene therapy, directed to HlV-1-seropositive multiple drug abusers because of the putative differential

role of CD45RA+ and CD45RA- CD4+ subpopulations in the generation of immunoregulatory cytokines. We also report a strong correlation between salivary and plasma levels of the TH1 cytokine, IL-2 among HIV-seropositive, gay/bisexual male methamphetamine abusers undergoing behavioral drug abuse treatment. We show that saliva provides as accurate an assessment of circulating IL-2 levels and of the IL-2/IL-4 ratio as plasma, and we propose that obtaining saliva from subjects where the collection of blood samples may be difficult provides a reliable body fluid for the assessment of certain fundamental measures of immune function.

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