Increased Immune Activation and Exhaustion in HIV-Infected Youth

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Abstract

Background—Immune activation and exhaustion drive several co-morbidities and disease progression in HIV-infected adults; however, they are not well-studied in HIV-infected youth. Thus, this study sought to examine levels of immune activation and exhaustion in this population, investigate associated HIV- and non-HIV-related variables, and compare results with a matched healthy control group.

Methods—HIV-infected youth 8–25 years old on stable antiretroviral therapy with an HIV-1 RNA level <1000 copies/mL were enrolled, along with matched healthy controls. We measured T-cell and monocyte immune activation and exhaustion markers in cryopreserved PBMC and plasma samples.

Results—136 subjects (80 HIV+: 66% male; 91% black) were enrolled. Markers of CD4+ and CD8+ T-cell activation were higher in the HIV-infected group vs. controls [mean % CD4+CD38+HLA-DR+ and CD8+CD38+HLA-DR+ = 2.2 vs. 1.5 (P=0.002) and 4.9 vs. 2.2 (P<0.0001), respectively], as were exhausted CD4+ and CD8+ T-cells [mean % CD4+CD38+HLA-DR+PD-1+ and CD8+CD38+HLA-DR+PD-1+ = 1.0 vs. 0.5 (P<0.0001) and 1.6 vs. 0.7 (P<0.0001), respectively]. There were no differences in proportions of inflammatory or patrolling monocytes between groups (P>0.05); however, soluble CD14 was higher in HIV-infected compared with controls (1.6 vs. 1.4 μg/mL; P=0.01). Current CD4 count, low-density

lipoprotein cholesterol, and age were the variables most associated with CD4+ and CD8+ T-cell activation.

Conclusions—CD4+ and CD8+ T-cell immune activation and exhaustion are higher in HIV-infected youth compared with matched controls, while monocyte sub-populations are not altered despite a high soluble CD14 level. The clinical significance of the increased immune activation and exhaustion should be further explored.

Keywords

HIV; immune activation; immune exhaustion; pediatrics

INTRODUCTION

HIV-infected individuals are living decades longer with the advent of combination antiretroviral therapy (**c**ART). Despite virologic suppression, however, they are still at an increased risk of co-morbidities, such as cardiovascular disease (CVD) and osteoporosis [1, 2]. While the etiology of these co-morbidities is multi-factorial, heightened immune activation (IA) plays an important role in the adult population [3–5]. Moreover, IA is associated with HIV disease progression, mortality, and poorer CD4+ T-cell reconstitution after initiation of **c**ART [6–9].

Data suggest that HIV-infected **y**outh are also at an increased risk of HIV-related co-morbidities like their adult counterparts [10–15]. Extrapolating from adult data would suggest that heightened IA would be an essential component of chronic HIV infection here too; however, it has not been well-characterized. Previous studies have investigated IA in this younger population [16–24]; however, there is limited prior research comprehensively investigated HIV-related and non-HIV-related factors associated with IA in HIV-infected youth.

In addition to heightened IA, immune exhaustion (IE) is also increased in HIV-infected adults suppressed on **c**ART [25]. One acceptable marker of IE is the increased expression of the inhibitory receptor, programmed cell death 1 (PD-1) on T-cells. Increased PD-1 expression levels predict the rate of HIV disease progression in adults [26, 27]. In addition, data suggest that IE could be critical in the viral persistence that characterizes chronic HIV infection [28]. To date, few studies have investigated IE in HIV-infected children [18, 23]. These studies both demonstrated an increase in IE compared to healthy controls, but only ART-naïve children were included.

Thus, the primary objective of this study was to comprehensively examine levels of IA and IE in HIV-infected youth on **c**ART with virologic suppression or low-level viremia. We hypothesized that similar to what is seen in HIV-infected adults, HIV-infected youth on **c**ART have increased levels of IA and IE. Secondary objectives included investigating HIV- and non-HIV-related variables associated with IA and IE in this population and comparing results to a matched healthy control group.

METHODS

Study Design/Population

This was a prospective, cross-sectional study investigating levels of IA and IE in HIV-infected youth on stable **c**ART and age-, sex-, and race-matched healthy controls. HIV-infected **youth** were recruited from the HIV clinics of University Hospitals Case Medical Center, Cleveland, OH and Grady Health System, Atlanta, GA via electronic medical record system queries and case manager/provider referrals. Subjects were eligible if they were between 8–25 years of age with documented HIV-1 infection on cART for 12 weeks prior to enrollment and with 6 months cumulative duration of cART and an HIV-1 RNA level <1,000 copies/mL. Exclusion criteria included any acute illness or inflammatory condition unless complete resolution 30 days prior to enrollment, malignancy, or medication use (*e.g.* chemotherapy agents, systemic steroids) which could affect results.

Controls were 8–25 years of age and healthy and were selected so that the group matched the HIV-infected subjects in regards to sex, race, and age. Controls were recruited in multiple ways, including a) friends or family members of the HIV-infected subjects, b) physician referrals from local pediatric and adult clinics, c) extensive outreach to various local organizations, churches, and schools, and d) recruitment flyers in targeted locations throughout the two cities. Both HIV-infected subjects and healthy controls were excluded from the study if they were pregnant or lactating.

The study was reviewed and approved by the Institutional Review Boards of University Hospital Case Medical Center, Emory University and Grady Health System. All parents or legal guardians and subjects 18 years of age gave written informed consent to participate in the study. Subjects aged 17 years of age signed a written consent along with their parent or legal guardian. Subjects between the ages of 8–10 years gave verbal assent and those 11–16 years gave written assent.

Study Assessments

Clinical evaluations—For HIV-infected subjects and healthy controls, relevant data were obtained by questionnaire, including demographics, current and past medical history, alcohol intake, tobacco use, and drug habits. Further information was also collected from the HIV-infected subjects' medical records including past and current medical diagnoses, CD4 nadir, detailed past and current antiretroviral (ARV) and non-ARV medication use, HIV diagnosis date, and acquisition method (perinatal or horizontal). Targeted physical examination, weight and height measurements were obtained in all subjects, and blood pressure was obtained with an automated, calibrated blood pressure machine.

Laboratory evaluations—After at least an 8-hour fast, blood was collected from all subjects for real-time measurements of insulin, glucose, and lipoprotein profile. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR; [fasting glucose (mg/dl)$\times$ fasting insulin (mU/ml)]/405) [29]. Absolute CD4 count and plasma HIV-1 RNA level were concomitantly measured as markers of HIV disease activity.

Additional whole blood was collected in EDTA tubes for immediate plasma and peripheral blood mononuclear cells (PBMCs) isolation and cryopreservation without prior thawing until analysis. For all laboratory assessments, laboratory personnel were blinded to clinical information and HIV status.

CD4+ and CD8+ T-cell and monocyte subsets as well as their respective levels of activation and exhaustion (for T-cells only) were assessed via flow cytometry. All flow cytometry staining was performed on the cryopreserved PBMCs that were thawed in a 37°C water bath and used immediately. Multi-parametric flow cytometric analysis was performed on these cells according to standard procedures, and the following panel of fluorochrome-labeled antibodies was used: anti-CD3-Alexa 700 (clone SP34-2), anti-CD8-allophycocyanin (APC)-Cy7 (clone SK1), anti-CD16-APC (clone 3G8), anti-CD38-phycoerythrin (PE) (clone HIT2), anti-CD86-fluroescein isothiocyanate (FITC) (clone FUN-1), and anti-HLA-DR-peridinin chlorophyll protein (PerCP)-Cy5.5 (clone G46-6) (BD Bioscience, San Jose, CA), anti-CD4-Pacific Blue (clone OKT4), and anti-CD20-Brilliant Violet 650 (clone 2H7) (Biolegend, San Diego, CA), anti-CD279 (PD-1)-PE-Cy7 (clone J105) (eBioscience, San Diego, CA), anti-CD14-ECD (RMO52) (Beckman Coulter, Brea, CA), and Live/Dead Fixable Aqua (Invitrogen, Carlsbad, CA).

Monocytes were identified by size and granularity, then as CD3−, CD20−, CD8−, CD4dim before being categorized into subsets by CD14 and CD16 expression: CD14+CD16− (classical), CD14+CD16+ (pro-inflammatory), and CD14dimCD16+ (non-classical/patrolling). T-cells were identified by size and granularity, as well as CD3+ and CD4+ or CD8+. T-cell activation and exhaustion levels were assessed by CD38, HLA-DR, and PD-1 expression, respectively. Flow cytometric acquisition was performed on an LSRII cytometer driven by FACS DiVa software and analyzed using FlowJo software (Treestar, Ashland, OR).

Monocyte activation was also assessed by measuring plasma concentrations of soluble CD14 (sCD14) with an enzyme-linked immunosorbent assay (ELISA) using Human CD14 DuoSet (R&D Systems, Minneapolis, MN). Median intra-assay and inter-assay coefficients of variance were <9%.

Statistical Considerations

Demographics and clinical and laboratory characteristics are described by study group, and HIV-related characteristics are described for the HIV-infected youth. Continuous measures are described using the appropriate measures of central tendency (mean or median) and dispersion (standard deviation (SD) or interquartile range (IQR)). Normally-distributed variables were compared using Student's *t*-tests, and non-normally-distributed variables were compared using Wilcoxon rank sum tests.

Correlations between the IA markers and variables of interest within the HIV-infected group were assessed using Spearman correlation coefficients for continuous variables. **Appropriate two-sample tests were used to assess marker differences in sub-groups for dichotomous variables (*e.g.* male vs. female).** Multiple regression analyses applying a backward elimination selection method was used to determine variables independently

associated with CD4 and CD8 activation. Variables were selected for inclusion in the regression analyses based on the results of the bivariate analyses.

Statistical analysis was conducted using SAS 9.2 (The SAS Institute, Cary, NC). Statistical significance was set at the 0.05 level.

RESULTS

Subject Characteristics

Study enrollment occurred from January 2012 to March 2014. A total of 136 subjects were enrolled, including 80 HIV-infected subjects and 56 healthy controls. By design, groups were well-matched for age, sex, and race (Table 1). The HIV-infected group had significantly more smokers, higher median systolic blood pressure and triglyceride level and a lower median high-density lipoprotein cholesterol level than the control group. None of the healthy controls were biologically-related to any of the HIV-infected subjects, and none had a history of *in utero* HIV/ARV exposure.

Levels of IA and IE

The mean (SD) of percent activated and exhausted CD4+ and CD8+ T-cells, as measured by co-expression of CD38 and HLA-DR and CD38, HLA-DR, and PD-1, respectively, was increased in the HIV-infected group compared to matched healthy controls (Figure 1A and 1B).

In contrast, the mean (SD) of percent CD14+CD16+ inflammatory and CD14dimCD16+ patrolling monocytes was similar between the HIV-infected and control groups (Figure 2A and 2B). However, the concentration of sCD14 was statistically higher in the HIV-infected group compared to the controls (Figure 2C).

Relationships between IA and Variables of Interest

Correlations between IA and variables of interest are described in Table 2. The most consistent non-HIV-related variable positively associated with both activated and exhausted CD4+ and CD8+ T-cells was age. The most consistent HIV-related variables negatively correlated with both CD4+ and CD8+ T-cell activation were ART and NRTI duration and current CD4 count. There were significant differences in both CD4 and CD8 activation and exhaustion markers when the following HIV-infected sub-groups were compared: male vs. female, HIV-1 RNA detectable vs. not detectable, and horizontal vs. perinatal transmission (Table 3).

Multivariable Regression Models

Using stepwise, backward elimination regression models, the variables most associated with both activated CD4+ and CD8+ T-cells included age, LDL cholesterol, and current CD4 cell count (Table 4). In the final models, only age and LDL cholesterol were statistically associated with activated CD4+ T-cells, and only age was statistically associated with activated CD8+ T-cells.

Sub-Group *Post-Hoc* Analysis

After the initial results were known, a *post-hoc* analysis was conducted to try to account for the variability of time that subjects may have been virologically-suppressed prior to study enrollment. A sub-group of HIV-infected subjects was created who had known sustained virologic suppression, which was defined as an HIV-1 RNA level <80 copies/mL for at 6 months prior to enrollment. Less than 80 copies/mL was chosen because there were several different assays used in the clinical laboratories to measure HIV-1 RNA levels with varying lower limits of detection (<20, <40, <48, <79 and <80 copies/mL). Less than 80 copies/mL was the highest cut-off among these assays to define undetectable HIV-1 RNA. We compared marker values for this sub-group (N = 46) to those of the healthy controls. CD8+ activation, CD4+ exhaustion, CD8+ exhaustion, patrolling monocytes, and sCD14 followed a similar pattern and magnitude to the results from the entire cohort. However, there was no longer a statically significant difference between groups for CD4+ activation (P = 0.43), and inflammatory monocytes were statistically higher in the controls (P = 0.03). Sustained virologic suppression was added as a variable to the multivariable regression of CD4+ activation, and it was not significant in the model (data not shown).

DISCUSSION

To our knowledge, this is the first study that comprehensively investigated levels of activated and exhausted T-cells and monocyte activation in HIV-infected youth compared to matched healthy controls. Importantly, we enrolled a well-characterized group of HIV-infected subjects that were on **c**ART with virologic suppression or low-level viremia to determine the effects of residual IA unrelated to ongoing active viral replication. We found that both activated and exhausted CD4+ and CD8+ T-cells were significantly higher in the HIV-infected group compared to the controls. Activated monocytes were more closely matched between groups, although sCD14 concentrations were statistically higher in the HIV-infected group compared to the levels in healthy controls.

In addition, an extensive investigation was undertaken to understand the variables most associated with IA and IE in this population. Arguably, current CD4 count was the most important HIV-related variable related to activated and exhausted T-cells. Age and LDL cholesterol appeared to be important non-HIV-related variables associated with activated CD4+ and CD8+ T-cells. There were fewer relevant variables associated with activated monocytes.

The increased percentage of activated CD4+ T-cells in HIV-infected children and young adults compared to healthy controls is consistent with other pediatric studies that investigated ART-treated, virologically-suppressed subjects [16, 17]. These previous reports conflicted about whether CD8+ T-cells are also increased in this population; however, the study Sainz, *et al* that did not show an increase, only evaluated 29 HIV-infected subjects and 9 controls. In our current study, with a larger sample size, ART-treated children and young adults have both an increased activated CD4+ and CD8+ T-cell population similar to adults [9, 30].

These findings may have serious implications for HIV-infected youth's quality of life. While cART dramatically restores health, HIV-infected individuals have more age-related co-morbidities than individuals in the general population [31]. This is partly due to the increased IA that persists despite **c**ART. In HIV-infected adults, T-cell activation is strongly predictive of disease progression and mortality in multiple studies [8, 9]. In addition, increased monocyte and T-cell activation is associated with HIV-related co-morbidities, such as CVD [3, 32]. It is generally unknown whether these relationships exist in HIV-infected youth, in whom chronic IA may be present for an entire lifetime (*i.e.*, for perinatally-infected youth).

In our study, current CD4 count was negatively associated with both activated CD4+ and CD8+ T-cells and remained in both final multivariable regression models. This is not a surprising finding given that in adult studies on-going IA is associated with poorer recovery of CD4 count despite effective **c**ART [9, 30, 33], and a higher frequency of activated CD8+ T-cells predicts a faster decrease of CD4 cells [34]. Thus, this association is an important finding for HIV-infected youth, as they will likely live with chronic HIV infection for many decades. Interventions aimed at decreasing residual IA should be investigated in this population. Statins, for example, have been shown to decrease levels of T-cell activation in HIV-infected adults [35].

We also saw an inverse relationship between LDL cholesterol and CD4+ and CD8+ T-cell activation (although only CD4+ activation was statistically significant in the multivariable regression model), where increased cell activation was associated with lower LDL cholesterol. This finding is consistent with previous studies showing that cytokines influence lipid and lipoprotein metabolism. For example, interferons decrease LDL cholesterol in non-HIV populations [36–38]. Thus, this inverse relationship likely represents levels of endogenous interferon activity (and other cytokines) which would be higher when CD4+ and CD8+ T-cell activation are increased.

Immune exhaustion is another feature of chronic HIV infection and is characterized by the expression of PD-1 on CD4+ and CD8+ T-cells. It is defined by loss of effector functions and proliferative capacity in memory T-cells [39]. The percent of IE was also increased in our study in the HIV-infected group compared to the healthy controls. This is consistent with adult studies [25] and one published pediatric study that investigated IE in solely ART-naïve subjects [18]. T-cell exhaustion was also associated with T-cell activation similar to the work in HIV-infected adults by the Pahwa group [40]. As with T-cell activation, T-cell exhaustion is predictive of HIV disease progression in HIV-infected adults [26, 27], and likewise should be further investigated in HIV-infected youth.

Interestingly, there was no statistically significant difference between groups for the percent of cells expressing the phenotype of activated monocytes. This may, however, be due to inadequate power, as concentrations of sCD14 were statistically higher in the HIV-infected group compared to the controls. The sCD14 concentrations found in this study among the HIV-infected youth were similar to other studies in both adults and pediatrics [16, 41].

Soluble CD14 is a marker of monocyte activation and independently predicts mortality in HIV-infected adults [7, 42, 43]. It is the receptor for lipopolysaccharide, a component of the cell wall of gram-negative bacteria, which circulates in the plasma after microbial translocation from a compromised intestinal mucosal barrier. These subsequent microbial products bind membrane or soluble CD14, contributing to IA [44, 45]. While this relationship has been clearly demonstrated in adults with HIV [43], only two studies have evaluated the relationship between sCD14 and gut microbial translocation in HIV-infected children. In both studies, the authors demonstrated a significant bivariate relationship between sCD14 and lipopolysaccharide levels [16, 18]. However, studies are needed in this younger population to investigate whether increased levels of sCD14 are predictive of mortality and/or disease progression in HIV.

Interestingly, sCD14 was positively correlated with cumulative duration of NNRTI and efavirenz use. This finding is consistent with a recent study that evaluated changes in plasma markers of inflammation and IA in ART-naïve HIV-infected adults initiating therapy with either elvitegravir/cobicistat/emtricitabine/tenofovir (EVG/c/FTC/TDF) or efavirenz/emtricitabine/tenofovir (EFV/FTC/TDF). In subjects who were in the EFV/FTC/TDF group, percent increases in sCD14 were seen at the 24- and 48-week time points, where both within-group and between-group changes were statistically significant [46]. The reason that EFV would cause an increase in sCD14 is unclear, however, and should be investigated further. Another interesting finding was that CD8+ activation and CD4+ exhaustion was higher in subjects who acquired HIV via horizontal transmission. These results follow the same pattern as ARV and HIV duration for CD8+ activation and ARV duration for CD4+ exhaustion, where the shorter the duration, the higher the marker. Thus, the significant relationship between horizontal transmission and CD8+ activation and CD4+ exhaustion is likely due to ARV and HIV duration as a reflection of virologic status, rather than mode of transmission *per se.* It should be pointed out, however, that neither ARV nor HIV duration was significant in the multivariable regression model with CD4+ activation.

There are limitations to this study. As a cross-sectional study, the associations demonstrated cannot prove causality, despite the potentially important findings. In addition, the subjects spanned a wide age range, including some younger and perinatally-infected subjects. However, this may be the population who would benefit the most from interventions which would decrease IA and IE, given the number of decades that they will live with HIV. Likewise, some of the HIV-infected subjects had low-level viremia, which may have affected the results. We tried to account for this possibility by conducting a sub-analysis including only subjects with sustained virologic suppression for at least 6 months prior to study enrollment. Results were similar for the majority of the markers with the exception of CD4+ T-cell activation and inflammatory monocytes. However, when sustained virologic suppression was included in the multivariable regression model with CD4+ T-cell activation, it was not significant. Thus, the lack of significance between the sub-group and the controls for CD4+ T-cell activation may be related to a smaller HIV-infected sample size vs. decreased (but not normal) CD4+ T-cell activation after 6 months of virologic suppression. It is unclear why the level of inflammatory monocytes in the HIV-infected sub-group was statistically lower than in the controls; however, there may be unmeasured confounders in this sub-group which would have to be further explored before drawing any conclusions.

Finally, this study was not powered and investigated a number of associations between IA and IE and variables of interest, which increases the risk of Type I errors. Nevertheless, these exploratory data provide a substrate from which to design future trials.

This study offers insight into IA and IE in HIV-infected youth. These results are novel as no previous pediatric study has comprehensively studied both IA and IE in **c**ART-treated subjects. Given the association with HIV disease progression, mortality, and the risk of HIV-related co-morbidities, such as CVD, in HIV-infected adults, it is imperative that we investigate this important topic in HIV-infected youth. Additional studies, particularly longitudinal trials, are needed to determine the long-term effect of increased IA and IE in this younger population, and serious consideration should be given to conducting randomized, placebo-controlled trials of potential interventions to minimize the negative effects of chronic IA and IE.

Acknowledgments

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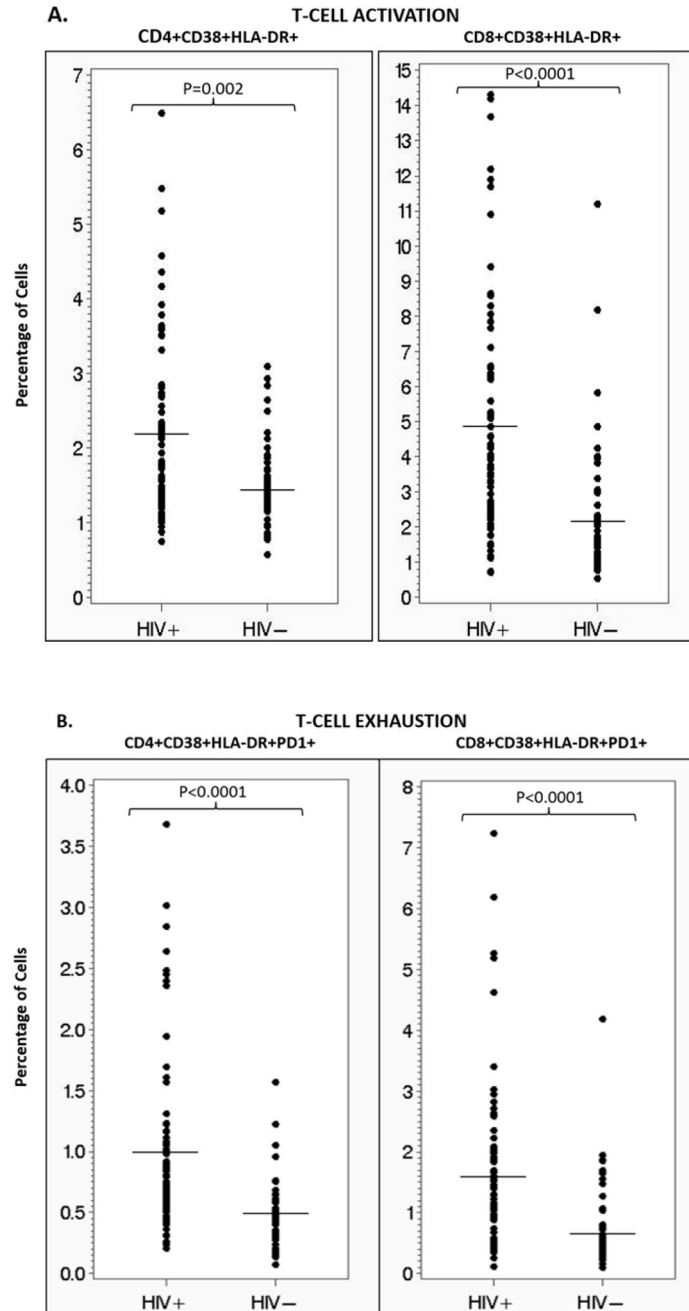
Figure 1. T-cell activation and exhaustion for HIV-infected subjects vs. controls
These scatter plots show the percentage of activated (A) and exhausted (B) CD4+ and CD8+ T-cells for the HIV-infected subjects compared to matched healthy controls. In all cases, the HIV-infected subjects have a significantly higher percentage of activated and exhausted CD4+ and CD8+ T-cells. Horizontal lines denote means. Mean % (standard deviation (SD)) CD4+CD38+HLD-DR+ was 2.2 (1.2) and 1.5 (0.6) for HIV-infected and controls, respectively. Mean % (SD) CD8+CD38+HLA-DR+ was 4.9 (3.3) and 2.2% (1.9) for HIV-infected and controls, respectively. Mean % (SD) for CD4+CD38+HLD-DR+-PD1+ was 1.0% (0.8) and 0.5% (0.3) for HIV-infected and controls, respectively. Mean % (SD) for

CD8+CD38+HLD-DR+-PD1+ was 1.6% (1.4) and 0.7% (0.7) for HIV-infected and controls, respectively.
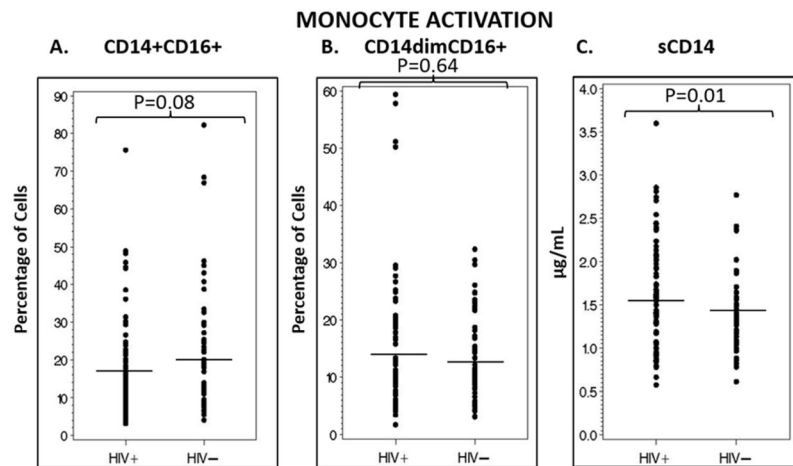
Figure 2. Monocyte activation for HIV-infected subjects vs. controls
These scatter plots show the percentage of inflammatory (A) and patrolling (B) monocytes, as well as the plasma concentration of sCD14 (C), a marker of monocyte activation, in HIV-infected subjects compared to healthy matched controls. Only sCD14 was significantly different between groups. Horizontal lines denote means. Mean % (standard deviation (SD)) CD14+CD16+ was 17.1% (13.1) vs. 21.6% (16.5), for HIV-infected and controls, respectively. Mean % (SD) CD14dimCD16+ was 14.3% (11.9) and 13.3% (7.6) for HIV-infected and control groups, respectively. sCD14 concentration was 1.6 (0.60) μg/mL and 1.4 (0.44) μg/mL for HIV-infected and control groups, respectively. sCD14, soluble CD14

Table 1

Subject Characteristics by Study Group

Median (Q1, Q3), or no. (%)	HIV-infected N = 80	Controls N = 56	P
Age, years	21 (17, 23)	18 (13, 23)	0.12
Male sex	53 (66)	28 (50)	0.08
Black race	73 (91)	46 (82)	0.12
Body mass index, kg/m^2	22.5 (19.6, 25.3)	22.0 (19.1, 27.5)	0.88
Systolic BP, mmHg	122 (114, 129)	110 (103, 121)	<0.01
Diastolic BP, mmHg	73 (68, 80)	69 (64, 77)	0.05
HDL cholesterol, mg/dL	47 (40, 56)	54 (45, 63)	<0.01
LDL cholesterol, mg/dL	87 (69, 112)	92 (69, 113)	0.96
Triglycerides, mg/dL	79 (64, 114)	70 (47, 87)	0.01
HOMA-IR	2.0 (1.4, 3.8)	2.1 (1.5, 3.0)	0.73
Current smoking	23 (29)	4 (7)	<0.01
HIV duration, years	6.5 (2.2, 14.5)	N/A	N/A
Current CD4 count, cells/mm^3	652 (421, 872)	N/A	N/A
CD4 nadir, cells/mm^3	290 (154, 424)	N/A	N/A
Undetectable HIV-1 RNA	63 (79%)	N/A	N/A
HIV-1 RNA level if not undetectable, copies/mL (N=17) [*]	89 (31, 590)	N/A	N/A
HIV-1 RNA <80 copies/mL	70 (88%)	N/A	N/A
HIV-1 RNA level if >80 copies/mL (N=10)	605 (143, 674)	N/A	N/A
Current ART use	80 (100%)	N/A	N/A
Current NNRTI use	33 (41%)	N/A	N/A
Current PI use	49 (61%)	N/A	N/A
Cumulative duration of ART, years	2.8 (1.2, 10.0)	N/A	N/A
Cumulative duration of NRTI, years	2.6 (1.2, 8.8)	N/A	N/A
Cumulative duration of NNRTI, years	0.6 (0, 2.2)	N/A	N/A
Cumulative duration of PI, years	1.8 (0.6, 6.6)	N/A	N/A
Perinatal transmission	36 (46%)	N/A	N/A
Prior AIDS diagnosis [**]	28 (35%)	N/A	N/A

[*] Because there were several different assays used in the clinical laboratories to measure HIV-1 RNA levels, there were varying lower limits of detection (<20, <40, <48, <79 and <80 copies/mL). <80 copies/mL was the highest cut-off that defined an undetectable HIV-1 RNA.

[**] As classified by the CDC 2008 Revised Surveillance Case Definition [47]

Q, quartile; BP, blood pressure; HDL, high-density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; N/A, not applicable; ART, antiretroviral therapy; NRTI, nucleoside analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; PI, protease inhibitor; CDC, Centers for Disease Control

Table 2

Correlations Between Variables of Interest and Markers of Immune Activation and Exhaustion in the HIV-infected Subjects

A.	% CD4+CD38+HLA-DR+[1]	% CD8+CD38+HLA-DR+[2]
Age, years	R=0.36 P<0.01	R=0.39 <0.01
LDL cholesterol, mg/dL	R=−0.29 P=0.01	R=−0.23 P=0.04
HDL cholesterol, mg/dL	R=−0.42 P<0.01	R=−0.24 P=0.04
HIV duration, years	NS	R=−0.27 P=0.02
ART duration, years	R=−0.33 P<0.01	R=−0.34 P<0.01
NRTI duration, years	R=−0.35 P<0.01	R=−0.34 P<0.01
Current CD4 count, cells/mm^3	R=−0.33 P<0.01	R=−0.33 P<0.01
% CD4+CD38+HLA-DR+	N/A	R=0.71 P<0.01
% CD4+CD38+HLA-DR+PD1+	R=0.89 P<0.01	R=0.75 P<0.01
% CD8+CD38+HLA-DR+PD1+	R=0.46 P<0.01	R=0.76 P<0.01
% CD14+CD16+	R=0.26 P=0.03	NS
% CD14dimCD16+	R=−0.42 P<0.01	NS
B.	**% CD4+CD38+HLA-DR+PD1+[3]**	**% CD8+CD38+HLA-DR+PD1+[4]**
Age, years	R=0.48 P<0.01	R=0.24 P=0.03
LDL cholesterol, mg/dL	R=−0.24 P=0.04	NS
HDL cholesterol, mg/dL	R=−0.37 <0.01	NS
ART duration, years	R=−0.38 P=0.04	NS
NRTI duration, years	R=−0.39 P<0.01	NS
Current CD4 count, cells/mm^3	R=−0.43 P<0.01	R=−0.3 P<0.01
% CD8+CD38+HLA-DR+PD1+	N/A	R=0.60 P<0.01
% CD14+CD16+	R=0.30 P=0.02	R=0.30 P<0.01
% CD14dimCD16+	R=−0.31 P<0.01	NS
C.	**% CD14+CD16+[5]**	**% CD14dimCD16+[6] sCD14[7]**

A.	% CD4+CD38+HLA-DR+[1]	% CD8+CD38+HLA-DR+[2]	
Waist-to-hip ratio	NS	R=−0.25 P=0.02	NS
NNRTI duration, years	NS	NS	R=0.33 P=0.03
Total use of EFV, years	NS	NS	R=0.31 P=0.01

[1] Mean % of CD4+ T-cells expressing CD38 and HLA-DR;

[2] Mean % of CD8+ T-cells expressing CD38 and HLA-DR;

[3] Mean % of CD4+ T-cells expressing CD38, HLA-DR, and PD-1;

[4] Mean % of CD8+ T-cells expressing CD38, HLA-DR, and PD-1;

[5] Mean % of monocytes expressing CD14+CD16+;

[6] Mean % of monocytes expressing CD14dimCD16+;

[7] Mean plasma concentration of sCD14

N.B. For continuous variables, Spearman Rank Correlation Coefficient was used to assess correlation. Number on top represents Spearman Correlation Coefficient and number on bottom represents P-value. **CD4 nadir also tested but did not have any significant relationships.** For each correlation between two immune activation/exhaustion markers, the inverse correlation is also true, but for simplicity, is listed only once.

NS, not significant; LDL, low density lipoprotein; HDL, high-density lipoprotein; ART, antiretroviral therapy; NRTI, nucleoside analogue reverse transcriptase inhibitor; N/A, not applicable; sCD14, soluble CD14; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; EFV, efavirenz

Table 3

Differences in Markers of Immune Activation and Exhaustion in Sub-groups among HIV-infected Subjects

	% CD4+CD38+HLA-DR+[1]	P*	% CD8+CD38+HLA-DR+[2]	P	% CD4+CD38+HLA-DR+PD1+[3]	P	% CD8+CD38+HLA-DR+PD1+[4]	P
Male sex	2.3 (1.2)		5.4 (3.3)		1.1 (0.9)		1.9 (1.5)	
Female sex	1.9 (1.2)	0.069	3.8 (3.0)	0.02	0.8 (0.6)	0.04	1.1 (0.8)	0.01
Detectable HIV-1 RNA	2.6 (1.1)		6.6 (3.6)		1.4 (1.0)		2.4 (2.0)	
Undetectable HIV-1 RNA	2.1 (1.2)	0.047	4.4 (3.0)	0.02	0.9 (0.7)	0.03	1.4 (1.0)	0.04
Horizontal Transmission	2.3 (1.1)		5.7 (3.3)		1.2 (0.8)		1.8 (1.5)	
Perinatal transmission	2.0 (1.2)	0.065	3.9 (3.0)	0.003	0.9 (0.8)	0.003	1.5 (1.2)	0.22

[1]Mean % (SD) of CD4+ T-cells expressing CD38 and HLA-DR;

[2]Mean % (SD) of CD8+ T-cells expressing CD38 and HLA-DR;

[3]Mean % (SD) of CD4+ T-cells expressing CD38, HLA-DR, and PD-1;

[4]Mean % (SD) of CD8+ T-cells expressing CD38, HLA-DR, and PD-1;

*
P-value represents the significance level for differences in mean % of T-cells expressing given phenotype for sub-group (*e.g.* male vs. female). Other sub-groups tested but were not significant include black race, current smoking, current marijuana use, current alcohol use, and previous AIDS diagnosis. There were no significant differences for % mean for CD14+CD16+ and CD14dimCD16+, or levels of soluble CD14 between any sub-group.

Table 4

Final Multiple Regression Models for Variables Associated with T-cell Activation in HIV-infected Subjects

A. CD4 Activation[1]

Variable	Beta	SE (Beta)	P
Age	0.0631	0.0285	0.03
LDL Cholesterol	−0.0081	0.0040	0.04
Current CD4 count	−0.0007	0.0005	0.12
		$R^2 = 0.19$	

B. CD8 Activation[2]

Variable	Beta	SE (Beta)	P
Age	0.1885	0.0764	0.02
LDL Cholesterol	−0.0190	0.0108	0.08
Current CD4 count	−0.0024	0.0012	0.06
		$R^2 = 0.20$	

[1] Mean % of CD4+ T-cells expressing CD38 and HLA-DR;

[2] Mean % of CD8+ T-cells expressing CD38 and HLA-DR

N.B. Other variables included in the initial models but eliminated using backward elimination selection method include sex, detectable HIV-1 RNA, ART duration, HIV duration

LDL, low-density lipoprotein; ART, antiretroviral therapy