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The effect of cellular isolation and cryopreservation on the expression of markers identifying subsets of regulatory T cells

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Abstract

Background—The role of CD4⁺ regulatory T cells (T_{regs}) and their subsets during HIV infection is controversial. Cryopreserved peripheral blood mononuclear cells (PBMC) are an important source for assessing number and function of T_{regs}. However, it is unknown if PBMC isolation and cryopreservation affect the expression of CD120b and CD39, markers that identify specific subsets of T_{regs}.

Methods—HIV-uninfected (HIV[−]) and -infected (HIV⁺) men were randomly selected from the Multicenter AIDS Cohort Study (MACS). Percentages of CD120b⁺ and CD39⁺ T_{regs} measured by flow cytometry in whole blood and in corresponding fresh and cryopreserved PBMC were compared.

Results—Percentages of CD120b⁺ T_{regs} were significantly lower in a) fresh PBMC relative to whole blood, and b) freshly thawed frozen PBMC relative to fresh PBMC when the recovery of viable cryopreserved cells was low. When present, low expression of CD120b in frozen PBMC was reversible by 4 hours of *in vitro* culture. In contrast, expression of CD39 on T_{regs} was not affected by isolation and/or cryopreservation of PBMC, or by relative recovery of cryopreserved PBMC. These findings were unaffected by the HIV status of the donor.

Conclusion—The data suggest that percentages of CD120b⁺ T_{regs} and CD39⁺ T_{regs} can be validly measured in either whole blood or PBMC (fresh and frozen) in HIV[−] and HIV⁺ men. However, for measurement of CD120b⁺ T_{regs} one type of sample should be used consistently within a given study, and thawed frozen cells may require *in vitro* culture if recovery of viable cells is low.

Keywords

CD4⁺ regulatory T cells; CD120b; CD39; PBMC cryopreservation; HIV

1. Introduction

Regulatory T cells (T_{regs}) are an important subset of CD4 T cells that suppress immune responses. T_{regs} are divided into different phenotypic and functional subsets (Simonetta and Bourgeois, 2013). For example, expression of CD120b (TNF- α receptor II) identifies a subset with high suppressive function (Mercer et al., 2010, Minigo et al., 2009, Chen et al., 2010). Another subset is defined by expression of CD39, an ecto-nucleoside triphosphate diphosphohydrolase, which facilitates the production of adenosine that inhibits T cell proliferation (Deaglio et al., 2007) and production of IL-2 (Jenabian et al., 2013). T_{regs} have been implicated in the control of immune responses under many physiological and pathological conditions, such as autoimmune diseases, allograft tolerance, tumors, and infectious diseases (Sakaguchi et al., 2010) including, controversially, HIV infection (Imamichi and Lane, 2012).

Cryopreservation of peripheral blood mononuclear cells (PBMC) is critical for assessing the phenotype and function of these cells in retrospective studies. Several studies have examined percentages of total T_{regs} before and after cryopreservation, with some showing lower percentages after cryopreservation (Elkord, 2009, Sattui et al., 2012) and others finding no difference (Van Hemelen et al., 2010, Venet et al., 2010, Shete et al., 2013). To our knowledge no study has evaluated the effect of cryopreservation on different subsets of T_{regs} . In addition, the process of PBMC isolation may also affect the expression of surface markers (Lundahl et al., 1995). Therefore, this study was undertaken to explore the effect of both PBMC isolation and cryopreservation on the expression of CD120b and CD39 on T_{regs} . PBMC from both HIV-uninfected (HIV-) and -infected (HIV+) men were analyzed, to test for an effect of HIV infection.

2. Materials and Methods

2.1. Study Population

HIV- and HIV+ men were randomly selected from the Baltimore-Washington, DC center of the Multicenter AIDS Cohort Study (MACS), an ongoing prospective cohort study of the natural and treated history of HIV-1 infection in men who have sex with men in Baltimore-Washington DC, Chicago, Los Angeles, and Pittsburgh (Detels et al., 2012). Briefly, the men are seen semi-annually, with each study visit including a medical and behavioral history; collection of blood for laboratory studies including HIV serology and (for HIV+ men) plasma HIV RNA concentration (viral load); and storage of serum, plasma and PBMC, as described (Detels et al., 2012). CD4 T cell counts were measured by flow cytometry as described (Hultin et al., 2007), and viral load was determined by the Roche ultrasensitive assay, which is sensitive to 50 copies/mL (Roche Diagnostics, Nutley, NJ). Because analyses of CD120b and CD39 expression were done in two separate time periods, two groups of subjects were studied.

2.2. Isolation, cryopreservation and thawing procedures of PBMC

PBMC were isolated by Ficoll Paque[®] density gradient centrifugation (GE Healthcare, Pittsburgh, PA) from sodium heparinized venous blood. Isolated cells were stained with trypan blue and counted using a Vi-CELL Series Cell Viability Analyzer (Beckman Coulter,

Indianapolis IN). Cryopreservation and thawing were performed as described (Aziz et al., 2013). 5 or 10×10^6 PBMC from each sample were suspended to 1×10^7 /ml in cold freezing solution (RPMI-1640 (Life Technologies, Carlsbad, CA) containing 50% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich Corporation, St. Louis, MO), and added to 2-ml cryogenic vials (Thermo Fisher Scientific Inc, Waltham, MA). The vials were kept overnight at -80°C in a freezing container (Nalgene Mr. Frosty, Thermo Fisher Scientific Inc), and transferred the next day to -140°C where they were stored for 1–12 months before analysis. On the day of analysis, frozen PBMC were thawed by immediate immersion of the cryogenic vials in a 37°C water bath with gentle agitation. The contents of the vial were then transferred to a 50-ml conical centrifuge tube and 10 ml of RPMI-1640 containing 50% FBS was added to the tube in a dropwise fashion. After centrifugation at 300g for 10 minutes, cells were washed once with RPMI-1640 containing 10% FBS, stained with trypan blue, and counted using a hemocytometer. The average recovery, calculated as the number of viable cells obtained from a vial divided by the number of PBMC initially frozen in the vial, was 80% ($\pm 20\%$ Standard deviation (SD)).

2.3. Immunophenotypic analyses and cell culture

Monoclonal antibodies (mAbs) were obtained from BD Biosciences, San Jose, CA, unless otherwise noted. To evaluate two phenotypes which identify T_{regs} cells (i.e., whole blood and corresponding fresh and frozen PBMC) were stained with anti-CD127–fluorescein isothiocyanate (FITC), anti-CD25–allophycocyanin (APC), and anti-CD4–allophycocyanin-Hilite[®]7 (APC-H7) for the phenotype $\text{CD4}^+\text{CD25}^+\text{CD127}^{\text{low/-}}$, and anti-FoxP3–Alexa Fluor[®] 488, anti-CD25–APC, and anti-CD4–Pacific Blue[®] for the phenotype $\text{CD4}^+\text{CD25}^+\text{FoxP3}^+$. To examine CD120b expression on T_{regs} ($\text{CD4}^+\text{CD25}^+\text{CD127}^{\text{low/-}}$), cells were stained with anti-CD127–FITC, anti-CD120b–phycoerythrin (PE), anti-CD25–APC, and anti-CD4–APC-H7. To examine CD39 expression on T_{regs} cells were stained with anti-CD25–PE, anti-CD4–Alexa Fluor[®] 700, anti-CD39–PE–cyanine 7 (Cy7), and anti-CD127–PE–Cy5 (eBiosciences, San Diego, CA). For whole blood, after incubating with the mAbs on ice in the dark for 30 minutes, erythrocytes were lysed by FACS Lysing solution (BD Biosciences) for 10 minutes at room temperature, and cells were washed twice with PBS and resuspended in FACS Lysing solution. The staining of PBMC, whether fresh or frozen, followed the same steps, except that no lysing step was performed. Intracellular staining of FoxP3 was performed according to the manufacturer's instructions, using the Human FoxP3 Buffer Set (BD Biosciences). Briefly, the cells were fixed for 10 minutes, followed by permeabilization for 30 minutes at room temperature in the dark. Then they were stained with anti-FoxP3 antibody on ice in the dark for 30 minutes. To examine the possibility of masking of CD120b immunostaining by $\text{TNF-}\alpha$ on freshly thawed PBMC, cells from HIV– donors were stained with higher than usual concentrations of anti-CD120b–PE (2 μg and 4 μg of antibody, versus 1 μg , per 1×10^6 cells).

For *in vitro* culture of PBMC, cells were resuspended at 1×10^6 /ml in RPMI-1640 containing 20% FBS and 1% antibiotic antimycotic solution (Sigma-Aldrich), plated at 1×10^6 /well in a 24-well plate (Sigma-Aldrich), and cultured at 37°C in 5% CO_2 in a humidified incubator. Changes in expression of CD120b and CD120a on frozen and fresh cells were monitored at

4, 8, 12, and 24 hours of culture. At each time point 1×10^6 cells were harvested, washed, and stained for immunophenotypic analysis with anti-CD3–Pacific Blue®, anti-CD120a–FITC, anti-CD120b–PE, anti-CD25–APC, and anti-CD4–APC–H7. Fresh PBMC were cultured in parallel with the frozen PBMC, harvested at the same time points, and stained with the same antibody panel for analysis.

For all flow cytometry analyses, fluorescence-minus-one controls (Roederer, 2002) were applied to set appropriate analysis thresholds. Fluorescence data were acquired on a FACSCanto II flow cytometer (BD Biosciences). Data were analyzed using FACSDiva software version 6.13 (BD Biosciences).

2.4. Statistical analysis

The non-parametric Wilcoxon signed rank test was used to compare paired data generated from whole blood, fresh PBMC, or frozen PBMC. The significance of differences between HIV– and HIV+ men was tested by Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Bivariate correlations among whole blood, fresh PBMC, or frozen PBMC were estimated using the Pearson correlation coefficient if the variable was distributed normally or log-normally, and the non-parametric Spearman correlation coefficient if not. To analyze the effect of HIV status on these correlations, HIV status was included in multiple linear regression analyses as an indicator variable. Analyses were performed with Stata version 11.2 (StataCorp, College Station, TX). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Subject characteristics

T_{regs} were studied for CD120b expression in 48 men (25 HIV–, 23 HIV+) and for CD39 expression in 34 men (21 HIV– and 13 HIV+). The characteristics of these donors are shown in Table 1a and 1b, respectively. Among the first group (Table 1a), HIV– and HIV+ men were similar in age, but HIV+ men were more likely to be of black ethnicity. Among the second group (Table 1b), HIV– men and HIV+ men were similar in ethnicity, but HIV+ men were younger. For both groups, most of the HIV+ men were virologically suppressed by antiretroviral therapy (ART).

3.2. Evaluation of T_{regs} assessed by the phenotypes $CD4^+CD25^+CD127^{\text{low/-}}$ and $CD4^+CD25^+FoxP3^+$

The percentages of T_{regs} among CD4 T cells were measured by two phenotypes, $CD4^+CD25^+CD127^{\text{low/-}}$ and $CD4^+CD25^+FoxP3^+$. By the phenotype $CD4^+CD25^+CD127^{\text{low/-}}$, these percentages were similar in whole blood and fresh PBMC for all but 2 donors (1 HIV– and 1 HIV+) (Fig. 1a). The same was true for the phenotype $CD4^+CD25^+FoxP3^+$ (Fig. 1b). Percentages of T_{regs} were significantly higher in HIV+ men than in HIV– men, as reported (reviewed in (Moreno-Fernandez et al., 2012)). Comparing fresh and frozen PBMC, percentages of T_{regs} assessed by the phenotype $CD4^+CD25^+CD127^{\text{low/-}}$ were similar (Fig 1c), but results for the phenotype $CD4^+CD25^+FoxP3^+$ were significantly lower in frozen PBMC (Fig 1d).

The percentages of T_{regs} measured by two phenotypes were highly correlated in whole blood ($r=0.73$, $p<0.01$), fresh PBMC ($p=0.59$, $p<0.001$), and frozen PBMC ($r=0.79$, $p<0.001$), for both HIV– and HIV+ donors (supplemental table 1). Therefore, the phenotype $CD4^+CD25^+CD127^{\text{low/-}}$ was valid for identifying T_{regs} in this study.

3.3. Comparison of CD120b expression on T_{regs} in whole blood and fresh PBMC

Percentages of $CD120b^+$ T_{regs} ($CD4^+CD25^+CD127^{\text{low/-}}CD120b^+$) among $CD4^+$ lymphocytes (Fig. 2a) and $CD120b^+$ among T_{regs} (Fig. 2b) were significantly lower in fresh PBMC than in whole blood. The significance of these differences was not affected by removing the three HIV+ men who had the greatest differences in Fig. 2a, or by HIV status (data not shown). Percentages of $CD120b^+$ T_{regs} in paired whole blood and fresh PBMC were highly correlated, and the slope of the relationship was not affected by HIV status (Fig. 2c). A significant correlation between whole blood and fresh PBMC was also observed in percentages of $CD120b^+$ cells among T_{regs} ($r=0.57$ (Spearman's correlation coefficient), $p=0.03$).

3.4. CD120b expression in freshly thawed and cultured thawed cells

Surprisingly, in the initial series of experiments on HIV– men, we found that the percentage of $CD120b^+$ cells among T_{regs} was very low in freshly thawed PBMC, whether T_{regs} were defined as $CD25^+CD127^{\text{low/-}}$ (Fig. 3a) or simply as $CD25^+$ (Fig. 3b, time 0) $CD4^+$ T cells. This low percentage was not increased by staining with higher concentrations of anti- $CD120b$ mAbs (data not shown), suggesting that it was probably not due to masking by free ligand. However, after overnight culture of the thawed cells $CD120b$ expression was increased (Fig. 3a), taking place within 4 hours (Fig. 3b) and reaching approximately the level seen on fresh T_{regs} (Supplemental Fig. 1a). Percentages of T_{regs} that were $CD120b^+$ among freshly thawed cells and thawed cells cultured for 4 hours were highly correlated ($r=0.73$, $p<0.01$ among $CD4^+CD25^+$ T cells). The same was true for thawed $CD8^+$ T cells, which also lost then regained $CD120b$ when thawed and cultured ($r=0.67$, $p=0.02$). $CD120b$ expression was low on non- T_{reg} $CD4^+$ T cells at all times, whether fresh or frozen (data not shown).

In a second series of experiments analyzing $CD120b$ expression on thawed PBMC from an additional 12 HIV+ men, recovery of viable cells was generally good (more than 70%) and $CD120b$ expression was similar on fresh and freshly thawed cells (0 hours in supplemental Fig. 1b). However, the percentages of $CD120b^+$ T_{regs} ($CD4^+CD25^+CD127^{\text{low/-}}CD120b^+$) in fresh and freshly thawed PBMC were poorly correlated ($r=0.35$, $p>0.05$). This correlation improved dramatically after 4 hours of culture ($r=0.78$, $p<0.01$, Fig. 3c). In these experiments, the 4 HIV– donors studied also demonstrated high recoveries and had no decline in $CD120b$ expression at any time after thawing (supplemental Fig. 2b).

Overall, the percentages of total T_{regs} did not change during *in vitro* culture (data not shown). For both T_{regs} ($CD4^+CD25^+$) and $CD8^+$ T cells, the percentage of $CD120b^+$ cells in freshly thawed PBMC was significantly correlated with the recovery of viable cells from the frozen PBMC ($r=0.42$, $p<0.01$ for $CD4^+CD25^+$ T cells, and $r=0.49$, $p=0.02$ for $CD8^+$ T cells). Culture of fresh PBMC did not affect $CD120b$ expression (data not shown). In

contrast to CD120b, expression of CD120a (TNF- α receptor I) was not affected by freezing, thawing, or *in vitro* culture (Supplemental fig. 3). During *in vitro* culture, the cell number and viability did not change significantly (data not shown).

3.5. CD39 expression on T_{regs} in whole blood, fresh PBMC, and frozen PBMC

The effect of cryopreservation on the expression of CD39 was evaluated in 34 men (21 HIV – and 13 HIV+), including 21 (10 HIV – and 11 HIV+) in whom the expression of CD39 in whole blood was also tested.

Percentages of CD39⁺ cells among T_{regs} were generally similar among whole blood, fresh PBMC, and frozen PBMC, although 2 HIV+ men showed very large differences between whole blood and fresh PBMC (Fig. 4a). Percentages of CD39⁺ cells in whole blood and fresh PBMC were highly correlated (Fig. 4b, $r=0.67$, $p<0.001$), as were those in fresh and frozen PBMC (Fig. 4c, $r=0.82$, $p<0.001$), even after adjusting for HIV status.

4. Discussion

This study assessed the effect of isolation and cryopreservation of PBMC on the expression of two markers of subsets of T_{regs}, CD120b and CD39, in HIV – and HIV+ men. Isolation of PBMC by density gradient centrifugation resulted in a decrease of CD120b expression on T_{regs}, and freezing-thawing of PBMC did the same when recovery of frozen cells was low, i.e., less than about 70%. When it occurred, the low expression of CD120b in frozen PBMC could be reversed after 4 hours of *in vitro* culture. Percentages of CD120b⁺ T_{regs} in whole blood and fresh PBMC were highly correlated, as were those in fresh PBMC and frozen PBMC that were cultured for 4 hours, in both HIV – and HIV+ men. Thus, for the purpose of group comparisons, percentages of CD120b⁺ T_{regs} can probably be measured validly using either whole blood or PBMC (fresh and frozen) in HIV – and HIV+ men, provided that one type of sample is used consistently.

The mechanism of reduced expression of CD120b on frozen T cells in samples with lower recovery of viable cells is unclear. Selective loss of CD120b⁺ cells is unlikely, because CD120b expression recovered rapidly in culture without apparent changes of cell numbers. A more likely possibility is stress-induced loss of CD120b, for two reasons. First, CD120b expression immediately after thawing was significantly and positively correlated with the recovery of viable cells. Second, factors leading to a low recovery may induce stress in the recovered viable cells; cellular stress has been reported to induce activation of the metalloprotease TNF- α converting enzyme (TACE) (Kuper et al., 2007) which in turn may lead to shedding of CD120b from T_{regs} (van Mierlo et al., 2008). However, TACE was also reported to be responsible for the shedding of CD120a (TNF- α receptor I) (Deng et al., 2015), and we found that the expression of CD120a on T_{regs} was not affected by freezing and thawing. Therefore, further studies are needed to explore the mechanisms of reduced levels of CD120b on freshly thawed T_{regs}.

In contrast to CD120b, CD39 expression on T_{regs} was similar and highly correlated among whole blood, fresh PBMC, and frozen PBMC. This was true regardless of HIV status. Therefore, CD39⁺ T_{regs} can be validly measured in all three types of sample. However, the

finding that two HIV+ men had very different CD39 expression in whole blood and PBMC raises the caution that PBMC isolation may affect CD39 expression in some HIV+ people, a phenomenon that requires further study.

Percentages of total T_{regs}, measured by the phenotype CD4⁺CD25⁺CD127^{low/-}, were not affected by cryopreservation of PBMC, which agreed with previous reports (Van Hemelen et al., 2010, Venet et al., 2010, Shete et al., 2013). These percentages were also well correlated with those measured by the phenotype CD4⁺CD25⁺FoxP3⁺, regardless of types of the samples and HIV status. These results suggest that the phenotype CD4⁺CD25⁺CD127^{low/-} can be used to identify T_{regs} in cryopreserved HIV+ samples.

Most of the HIV+ men studied here were treated with ART and had very low or undetectable plasma HIV RNA concentrations. Therefore the results in this study may not apply to HIV+ people who are not virologically suppressed, or to women. However, the results should apply to studies in HIV- men or men living with long-term suppressed HIV infection, for example on the role of T_{regs} in chronic diseases (Tan et al., 2014), inflammation (Phetsouphanh et al., 2015), and aging (Zhang et al., 2015).

5. Conclusion

In the present study, we validated the measurement of the percentages of CD120b⁺ T_{regs} and CD39⁺ T_{regs} in either whole blood or PBMC (fresh and frozen) in HIV- and HIV+ men. Because CD120b expression on T_{regs} was reduced by isolation and cryopreservation when the recovery of cryopreserved cells was low, one type of sample should be used consistently within a given study, and thawed frozen cells may require *in vitro* culture if recovery of viable cells is low.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig.1.

Percentages of T_{regs} among CD4 T cells measured by the phenotypes CD4⁺CD25⁺CD127^{low/-} and CD4⁺CD25⁺FoxP3⁺, in paired whole blood (WB), fresh PBMC, and frozen PBMC from HIV- and HIV+ men. Data were obtained from HIV- men (filled circles, solid lines) and HIV+ men (open circles, dotted lines), using the phenotypes CD4⁺CD25⁺CD127^{low/-} (a and c) and CD4⁺CD25⁺FoxP3⁺ (b and d), for WB and fresh PBMC (a and b) and fresh and frozen PBMC (c and d). The thick lines connect the median percentages in each group. The percentages in WB and fresh PBMC were similar when measured by CD4⁺CD25⁺CD127^{low/-} (a) or CD4⁺CD25⁺FoxP3⁺ (b), although two men (marked in red) showed greater changes than the others (a). The percentages were significantly lower in frozen PBMC than in fresh PBMC in d but not in c.

Fig. 2.

Expression of CD120b on T_{regs} in paired whole blood and fresh PBMC from HIV- and HIV+ men. a and b. Comparisons of percentages of (a) CD120b⁺ T_{regs} among CD4⁺ lymphocytes and (b) CD120b⁺ cells among T_{regs} in whole blood (WB) and fresh PBMC for HIV- men (filled circles, solid lines) and HIV+ men (open circles, dotted lines). The thick line connects the median percentages in WB and fresh PBMC. Differences between WB and fresh PBMC were significant in both a and b. In b, removing the three HIV+ men who had the greatest differences (marked in red) did not affect the significance of this difference. c.

Correlations of percentages of CD120b⁺ T_{regs} in WB and fresh PBMC by HIV status. The line for the entire group is derived from an unadjusted least-squares regression and shows a strong correlation ($r=0.82$, $p<0.0001$). The lines shown for HIV– men and HIV+ men are derived from multiple linear regression analysis adjusting for HIV status. In the equations, y represents the percentage of CD120b⁺ T_{regs} in fresh PBMC, and x the percentage of CD120b⁺ T_{regs} in WB. The slopes of these did not differ significantly.

Fig. 3.

Changes of CD120b expression in frozen PBMC during *in vitro* culture. a. The gating strategy is shown beginning at the upper left: after identifying CD4⁺ T cells, CD25⁺CD127^{low/-} cells were identified, and the changes of CD120b expression on these cells were measured (right panels). Expression of CD120b on T_{regs} thawed freshly and those cultured overnight from a representative donor are shown and were lower immediately after thawing (top right, 8.6%) than after overnight culture (bottom, 44.7%). b. Percentages of CD120b⁺ cells among T_{regs} (CD4⁺CD25⁺) in frozen PBMC from HIV⁻ men. Percentages were low immediately after thawing (0 hours), rose substantially after 4 hours of culture, and then remained nearly constant throughout 24 hours. Lines indicate data obtained from the same donor. The bold line indicates the median percentages at the time points. c. Relationships of percentages of CD120b⁺ T_{regs} (CD4⁺CD25⁺CD127^{low/-}CD120b⁺) in fresh and frozen PBMC from HIV⁺ men. The frozen PBMC were cultured for 4 hours. The line represents the least-squares regression line of the data. In the equation, y represents the percentage of CD120b⁺ T_{regs} in fresh PBMC, and x represents the percentage of CD120b⁺ T_{regs} in frozen PBMC.

Fig. 4.

Percentages of CD39⁺ cells among T_{regs} in WB, fresh and frozen PBMC. a. Comparisons of percentages CD39⁺ cells among T_{regs} obtained from HIV⁻ men (filled circles, solid lines) and HIV⁺ men (hollow circles, dotted lines). The bold line connects the median percentages in the three types of samples. The red dots and lines denote two HIV⁺ men who had large differences between WB and fresh PBMC (−67% and +31%, respectively). b and c. Relationships of CD39 expression in WB, fresh PBMC, and frozen PBMC. Percentages of CD39⁺ cells among T_{regs} in fresh PBMC (vertical axes) are shown in relation to the

corresponding percentages in WB (b) and frozen PBMC (c). The lines for all men (i.e., combined HIV– and HIV+ men) were derived from unadjusted least-squares regressions and show strong correlations ($r=0.67$, $p<0.001$ for b; $r=0.82$, $p<0.001$ for c). The separate lines for HIV– men and HIV+ men were derived from multiple linear regression analysis adjusting for HIV status. In the equations, y represents the percentage of CD120b⁺ T_{regs} in fresh PBMC, and x the percentage of CD39⁺ T_{regs} in WB (b) or frozen PBMC (c). In both cases, the slopes of these lines did not differ significantly by HIV status.

Table 1

Demographic and Laboratory Characteristics of Study population

a. For analysis of CD120b	HIV-Negative	HIV-Positive	Total
N	25	23	48
Ethnicity			
Black	4 (16) ^{*†}	16 (70)	20 (42)
White	21 (84)	7 (30)	28 (58)
Age, median (IQR) ^{**}	58 (52–62)	57 (50–61)	57 (51–62)
CD4 cell count (/μL), median (IQR)	985 (796–1117) [†]	630 (536–941)	812 (604–1076)
Antiretroviral treatment (ART)		19 (82)	
Undetectable viral load (<50 copies/ml)		17 (74)	
b. For analysis of CD39			
N	21	13	34
Ethnicity			
Black	4 (19)	5 (38)	9 (26)
White	17 (81)	8 (62)	25 (74)
Age, median (IQR) ^{**}	59 (56–69) [†]	54 (44–59)	59 (53–66)
CD4 cell count (/μL), median (IQR)	1269 (1119–1417) [†]	974 (797–1287)	1214 (974–1413)
Antiretroviral treatment (ART)		12 (92)	
Undetectable viral load (<50 copies/ml)		10 (77)	

* Data are number (percentages) unless otherwise indicated.

** IQR= interquartile range (25th–75th percentiles)

[†] p<.05 for comparison between HIV– and HIV+ men.