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Normal T cell activation in Elite Controllers with Preserved CD4 T cell counts

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Abstract

Background—HIV Elite controllers (EC) suppress HIV viremia without ART, yet previous studies demonstrated that EC maintain an activated T cell phenotype. Chronic immune activation has detrimental consequences and thus ART has been advocated for all EC. However, EC is not a clinically homogenous group. Since CD4% is among the best predictors of AIDS related events, in the current study, we assessed whether this marker can be used to stratify EC needing ART.

Methods—Sixteen EC were divided into 2 groups based on CD4% (EC^{hi}>40% and EC^{lo} 40%), and T cell subsets were analyzed for markers of memory/differentiation (CD45RA, CCR7, CD28), activation (CD38/HLA-DR), immunosenescence (CD57), co-stimulation (CD73, CD28) and exhaustion (PD-1, CD160, Tim-3). Monocyte subsets (CD14, CD16) were also analyzed and sCD14 levels were quantified using ELISA.

Results—In the EC^{hi} group, expression of activation, exhaustion, and immunosenescence markers on T cells were significantly reduced compared to the EC^{lo} group and similar to the seronegative controls. The EC^{hi} group expressed higher levels of co-stimulatory molecules CD28 and CD73 and had lower levels of monocyte activation (HLA-DR expression) with a reduced frequency of inflammatory monocyte (CD14++CD16+) subset. Furthermore, the EC^{hi} group maintained a stable CD4% during a median follow up of six years.

Conclusions—Elite controllers with preserved CD4 T cells (EC^{hi}) have normal T cell and monocyte phenotypes and therefore may have limited benefit from antiretroviral therapy (ART).

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CONFLICT OF INTEREST:

All authors declare no financial conflict of interest for this study.

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CD4% can be an important marker for evaluating future studies aimed at determining the need for ART in this group of individuals.

Keywords

HIV; elite controllers; immune activation; CD4 counts; CD4%; immune exhaustion; T cells

INTRODUCTION

A minority of HIV infected individuals (less than 1% of the total HIV infected population) control HIV with low or undetectable plasma viral loads (pVL) in the absence of antiretroviral treatment (ART). These rare individuals, identified as elite controllers (EC), represent an excellent *in-vivo* model of spontaneous HIV control [1, 2].

Published data suggest that EC maintain durable viral control through an armamentarium of virological, immunological and genetic features [1, 3, 4]. Notably, polyfunctional T cells, capable of cytokine secretion, proliferation, and cytotoxicity are maintained in these controllers [4–9]. Additional important aspects of their T cell function have been reported, including low levels of T-cell activation [10], up regulation of survival factors such as bcl-2 [11], up regulation of cyclin dependent kinase inhibitors such as p21 [12] and higher frequency of expression of costimulatory molecule CD73 [13]. Furthermore, cells from EC are less susceptible to infection and effectively suppress *in-vitro* HIV replication [8, 14]. The EC group is also enriched for HLA-B*27 and B*57 alleles that have been associated with protection from HIV disease progression [15, 16].

Despite these advantages, EC fail to clear HIV infection. Ultra-sensitive assays measuring plasma viral load (pVL) indicate that residual pVL remains higher in some EC than in ART treated individuals [17, 18]. Additionally, CD4 T cells from EC can harbor pathogenic replication competent virus that may contribute to excess T cell activation [19, 20].

Interestingly a recent SIV study suggested that effector CD8 T cells are unable to access B cell follicles and target infected T follicular helper cells. The latter probably serves as a viral reservoir responsible for the low level and persistent antigenemia [21]. Alterations in innate immune function have also been reported in EC with increased inflammatory monocytes compared to HIV uninfected controls [22]. Recently, EC have also been shown to have elevated levels of key soluble inflammatory markers [23] and higher hospitalization rates than ART treated individuals [24].

Up regulation of T cell immune activation markers (CD38, HLA-DR and/or Ki67) is a well described feature of chronic HIV infection [10, 25, 26] linked with disease progression [10, 27]. It is likely that low level viremia is one factor contributing to elevated innate and adaptive immune activation in EC that remains higher than HIV-1 seronegative and ART treated individuals [10, 18]. However, even with suppressive ART, key markers of immune activation are not reduced to levels that are on par with HIV-1 seronegative individuals. As a consequence, despite being on ART, HIV infected individuals are at increased risk of inflammation associated co-morbidities [10, 28, 29].

Since persistent immune activation and the ensuing inflammation can have detrimental consequences, it has thus been suggested all EC may benefit from ART [19, 30]. However, HIV controllers are not a homogenous group [31–34] whereby some individuals maintain absolute CD4 counts over time whereas others display activation induced loss [10, 35, 36]. Furthermore, a prior study demonstrated an inverse correlation between absolute CD4 T cell counts and T cell immune activation [10] suggesting a dynamic range in immune activation within HIV controllers. A discriminatory marker to identify ECs who are more likely to have persistent immune dysfunction would aid clinicians in assessing who may benefit the most from early ART initiation.

CD4% has been used to stratify individuals needing ART since it is among the best predictors of AIDS related events [37–40]. We, therefore, assessed expression levels of immune activation/exhaustion markers on T cells based on the CD4% of HIV infected patients. EC with preserved CD4 T cell percentages (EC^{hi}) had significantly lower levels of activated/exhausted HIV-specific CD8 T cells than EC with low CD4 T cell percentages (EC^{lo}) and the former mirrored the seronegative controls. Based on these results, EC^{lo} may benefit the most by initiating ART or other inflammation reducing treatments while no evidence was obtained to suggest that such treatments would benefit the EC^{hi} group.

MATERIALS AND METHODS

Study cohort

Cryopreserved PBMC and plasma samples from 25 HIV-1 infected patients receiving care at the 1917 clinic at the University of Alabama at Birmingham and 8 HIV-1 seronegative (SN) volunteers enrolled in the AVRC clinic at UAB were used in this study. All HIV infected patients were off ART. The elite controllers (EC) used in this study met the criteria of consecutive undetectable HIV-RNA measurements for greater than six months or with greater than 90% of these measurements being less than 400 copies/ml over several years as suggested by a prior study [41]. The EC were divided into two groups based on their longitudinal CD4% over a median of 6.2 years of follow-up. These were EC^{hi} (pVL low to undetectable, median CD4% >40, n=9) and EC^{lo} (pVL low to undetectable, median CD4% 40, n=7). We compared the two elite controller groups against 2 comparator groups i.e. HIV seronegative (SN) and HIV-1 non-controllers (pVL>10,000 copies/ml, n=9, NC). Local institutional IRB approval was obtained and all participants consented to this study.

Polychromatic flow cytometry

Cryopreserved PBMC were thawed and surface stained using fluorochrome conjugated antibodies in three different panels in parallel to assess ex-vivo expression levels of activation, exhaustion, immunosenescence and memory markers. The cells were stained with LIVE/DEAD cell dye (Invitrogen), washed, and surface labeled with the following antibodies: **Panel 1** – anti-CD3 (APC-eFluor 780, eBioscience), anti-CD4 (Qdot655, Invitrogen), anti-CD8 (V500, BD), anti-CD45Ra (Brilliant Violet 421, BD), anti-CCR7 (PerCPy5.5, BD), anti-CD28 (PE, BD), anti-CD57 (FITC, BD), and anti-PD1 (APC, eBioscience); **Panel 2** – anti-CD3, anti-CD4, anti-CD8, anti-CD38 (PE, BD), anti-HLADR (PerCPy5.5, BD), anti-CD73 (PECy7, BD), anti-PD1, anti-CD160 (Alexa488, BD), and

anti-TIM3 (Brilliant Violet 421, Biolegend); **Panel 3** – anti-CD3, anti-CD14 (Alexa700, BD), anti-CD16 (FITC, BD), anti-HLADR, anti-CCR2 (Alexa647, BD), anti-CX3CR1 (PE, BD), and anti-CD11b (PECy7, BD). CCR7 staining was performed first by incubating cells at 37°C for 15 minutes, followed by washing and staining for all other surface markers for 20 minutes at 4°C. Samples were washed, fixed in 2% paraformaldehyde and analyzed on a BD flow cytometer (LSR II). At least 100,000 CD3+ events were acquired and the data were analyzed using FlowJo software (version 9.7, Tree Star Inc). Frequency and mean fluorescence intensity (MFI) data were analyzed and compared for all single markers. Boolean combination gating was performed to analyze combinations of 3 or more markers.

Soluble CD14 ELISA

Plasma levels of sCD14 were quantified using a Human sCD14 Quantikine® ELISA kit (R&D Systems, Minneapolis, MN). Briefly, plasma samples were prefiltered, UV inactivated, diluted 1:200 and incubated in plates pre-coated with anti-human sCD14 antibody for 3 hours. After washing, plate-bound sCD14 was detected with an HRP-conjugated anti-sCD14 antibody for 1 hour. The plate was developed using TMB for 30 minutes before 2N H₂SO₄ stop solution was added. Plate-bound sCD14 was quantified by measuring the absorbance at 450nm with a wavelength correction at 540nm using a BioTek Synergy HT reader. Plasma levels of sCD14 were interpolated from the standard curve using Microsoft Excel software.

Statistical analyses

For the categorical variables, both chi-square and Fisher's exact tests were performed. For continuous variables the non-parametric Kruskal-Wallis test was used to assess differences among the four groups. The expression of markers between the groups was compared pairwise using the non-parametric Mann-Whitney Test and covariation between two variables was quantified using the non-parametric Spearman correlation. Clinical data (CD4% and absolute CD4 count) for each individual at multiple longitudinal time points were obtained. The mean values for each of the above clinical data for the EC^{hi} and EC^{lo} groups at each time point were graphed and fit with a linear regression line. The slopes of the regression lines were compared using ANCOVA. The overall change in these two measures from the first to last available time points was also calculated and the medians for each group were compared using non-parametric Mann-Whitney Test. All analyses were performed using Prism software (version 6, GraphPad Software Inc).

RESULTS

Heterogeneity in the frequency and magnitude of CD4 T cells in the elite controller group

We categorized two EC groups based on the median CD4% for each individual and whether that individual maintained their categorical CD4% over time. We chose 40% CD4 as a cut-off since the median of the EC group as a whole was 41% and designated the two groups as EC^{hi} (CD4% >40) and EC^{lo} (CD4% ≤ 40).

After a median follow up of 6.2 years, clinical data revealed clear heterogeneity between the two EC groups (figure 1) with respect to absolute CD4 counts and CD4%. While there was

significant overlap for the median absolute CD4 T cell count for both EC^{hi} and EC^{lo} groups, there were distinct differences in the median CD4 percentage, as expected (figure 1 A and B). While most individuals demonstrated relatively stable CD4% and absolute count over time (figure 1C and D), there was a trend for a decrease in CD4% (figure 1C, $p=0.067$) in the EC^{lo} group compared to the EC^{hi} group.

Furthermore, pVL was lower in the EC^{hi} group and with fewer intermittent viral blips compared to the EC^{lo} group (supplementary figure 1). Given these clinical distinctions among the two EC groups, we evaluated whether a higher CD4% equated with lower levels of expression of cellular markers of activation and exhaustion.

The demographic and clinical features of the study subjects in each group are shown in Table 1. Most HIV infected subjects were African-American who acquired HIV through heterosexual or MSM transmission. The four groups were comparable to each other except for the race and age and the latter was not different when comparing the two EC groups. Detailed demographic and clinical characteristics for each study participant are shown in supplementary table 1. One individual in each group (subject 9 and 16) was evaluated for immunogenicity at two different time points (Table 1 and supplementary table 1).

Reduced T cell activation in the EC^{hi} group

Since immunologic markers such as CD38 and HLA-DR have been shown to be predictive of HIV disease progression [42, 43], we assessed their surface expression in each of the 4 groups of subjects. A comparative analysis of the EC groups revealed that the EC^{hi} CD8 T cells expressed lower HLA-DR (median 5% vs 33%, $p<0.0001$) and CD38+HLA-DR+ (2% vs 17%, $p=0.0002$) compared to the EC^{lo} group (figure 2A). Similar data were also observed in the CD4 T-cells (figure 2B). Additionally, for CD8 T cells, a trend for lower expression of CD38 for the EC^{hi} was observed (23% vs 38%, $p=0.058$; figure 2A). For both the immune activation markers, EC^{hi} were indistinguishable from the seronegatives with regards to the CD8 and CD4 T cells. Additional differences in marker expression were also seen when either of the EC groups were compared to the SN and NC (supplementary table 2 and 3).

An inverse correlation was previously reported between absolute CD4 counts and immune activation of T cells [10]. Similarly, in our EC cohort, negative correlations were observed between CD4% and CD38+ HLA-DR+ expression on CD8 T cells ($r=-0.8306$, $p<0.0001$; figure 2C) and CD4 T cells ($r=-0.7742$, $p=0.0002$; figure 2D). Correlations between CD8 T cell activation and CD4% were not observed for the NC group (data not shown).

Lower frequency of terminally differentiated CD8 T cells in the EC^{hi} group

The proportion of T cells in various stages of maturation can be perturbed by alterations in viral load [44]. To evaluate whether CD4% impacted skewing of these populations, we characterized CD4 and CD8 T cell memory subsets by surface expression of CCR7, CD45RA and CD28 and defined as naïve (CCR7+ CD28+CD45RA+), central memory (CCR7+CD28+CD45RA-), effector memory (CCR7-CD28 +/-CD45RA-), and CD45RA+ effector memory or Temra (CCR7-CD28-CD45RA+). Regarding the phenotype of CD4 T cells, SN, EC^{hi}, and EC^{lo} had significantly higher frequency of central memory T cells than

non-controllers ($p=0.0002$; $p=0.036$; $p=0.050$) respectively; data not shown. For the CD8 T cells, the EC^{hi} group had a significantly lower frequency of CD8 T cells that displayed the Temra phenotype compared to the EC^{lo} group ($p=0.03$, supplementary figure 2). Additionally, the EC^{hi} group had higher frequency of naïve CD8 T cells compared to EC^{lo} ($p=0.05$). Therefore, overall, the EC^{hi} had a less differentiated CD8 T cell phenotype than the EC^{lo} group.

Reduced expression of exhaustion markers PD1 and CD160 on T cells in the EC^{hi} group

We evaluated the expression of three exhaustion markers that are commonly used to determine this phenotype i.e. PD-1, CD160 and TIM-3 [45]. The EC^{hi} had decreased PD-1 (20% vs 30%, $p=0.0418$), CD160 (21% vs 63%, $p=0.003$) and CD160, PD1 (5% vs 17%, $p=0.003$) compared to EC^{lo} on the CD8 T cells (Figure 3A and C). A similar pattern of PD1 and CD160 expression was also seen in CD4 T cells ($p=0.006$) (Figure 3B and D). The EC^{hi} also had lower MFI of PD-1 on CD8 (4297 vs. 4854, $p=0.006$) and CD4 T cells (4641 vs. 5041, $p=0.04$) compared to EC^{lo}. Details of other marker combinations that were different between all groups are shown in supplementary table 2 and 3.

Lower expression of immunosenescent (CD57) and increased expression of costimulatory molecules (CD28 and CD73) on CD8 T cells in the EC^{hi} group

Persistent viral infections such as HIV lead to increased accumulation of cells that display a CD28[−] and CD57⁺ phenotype due to persistent antigenic stimulation. [46–48]. We therefore assessed whether the T cells in the EC^{hi} group had a higher proportion of functionally responsive cells. As expected, CD8 T cells from the EC^{hi} revealed a phenotype of decreased T cell senescence and increased costimulatory molecule expression. The EC^{hi} CD8 T cells had markedly lower frequency of CD57 (median 22% vs 45%, $p=0.004$), higher CD28 (80% vs 36%, $p=0.003$), higher CD28⁺, CD57[−] (71% vs 27%, $p=0.0003$), and higher CD73 (36% vs 8%, $p=0.005$) than EC^{lo} (Figure 4A). In each case, EC^{hi} were similar to seronegatives, whereas the EC^{lo} were comparable to non-controllers. Additional data for the inter- group comparisons is shown in supplementary table 2 and 3.

A prior publication showed an inverse correlation between CD73 expression and CD8 T cell activation [13]. We too observed a negative correlation for the combined EC group which was most likely driven by the EC^{hi} group (figure 4B). However, we did not see a correlation for either the SN or NC although there was a trend in the latter group (figure 4C and D).

Lower frequency of circulating inflammatory monocytes in the EC^{hi} group

Monocyte subpopulations were defined into classical (CD14⁺⁺CD16[−]), intermediate / inflammatory (CD14⁺⁺CD16⁺), and non-classical / patrolling (CD14⁺CD16⁺⁺) as previously described [49]. The EC^{hi} subgroup showed decreased frequency of intermediate / inflammatory monocytes compared to the EC^{lo} (median 1% vs 2.2%, $p=0.001$, supplementary figure 3). This inflammatory subset also expressed lower median levels of HLA-DR when compared to EC^{lo} (95.7% vs. 98.3%, $p=0.033$); data not shown. The frequency and activation levels for the other two monocyte subsets were similar among the EC groups; supplementary figure 3. Plasma levels of sCD14 were not different among the two EC groups or the EC overall group compared to SN and NC.

DISCUSSION

Antiretroviral therapy (ART) has significantly improved over the past several years allowing administration of potent, simple regimens that are well tolerated and associated with minimal side effects. However, even patients with asymptomatic HIV infection and normal absolute CD4 T cell counts have evidence of immune activation, a parameter associated with disease progression [10]. This information has led many providers to recommend treatment for all HIV positive patients regardless of their CD4 T cell counts [50]. Elite controllers, who appear to maintain control of their HIV, are a rare group of individuals for whom the risks of ART may outweigh the benefits, as the latter may not be obvious without candid evidence of disease progression. Nevertheless, several studies have demonstrated that EC also have evidence of immune activation and may benefit from ART as well. Such prior studies have generally considered EC to represent a single group; however, such patients actually demonstrate heterogeneity- clinically, genetically, and immunologically [32–36]. In this study, despite the small sample size of the EC cohort, we have shown that CD4% is an easily quantifiable benchmark for gauging the clinical heterogeneity in the EC group as reflected by the stark differences in the immune phenotypes observed. Specifically, our data demonstrated that CD8 T cells from EC with high CD4% (EC^{hi}) have expression levels of markers of immune activation, senescence, and exhaustion that are comparable to HIV seronegative controls and decreased when compared to HIV-infected patients including EC with low CD4% (EC^{lo}) and non-controllers (NC). Future studies will assess the clinical outcome from ART in EC with low CD4%.

Some of the heterogeneity in EC may stem from the varied clinical definitions that have been used to identify EC [51]. As recommended in a recent review of EC literature, we used the criteria of EC having either consecutive undetectable HIV-RNA measurements for greater than six months or with greater than 90% of these measurements being less than 400 copies/ml over several years [41]. Although the pVL is largely undetectable in our two EC groups, viral blips were less frequently observed in the EC^{hi} group. Hence it is possible that a lack of these episodes of intermittent viremia may contribute to the increased frequency of naive and lower proportion of terminally differentiated cells seen in the EC^{hi} group. Since viral blips [52] have also been associated with activated CD8 T cells, it is not surprising that the T cells in the EC^{hi} group are less activated and in part responsible for the preserved CD4% over time. Thus, only the EC^{lo} may benefit from ART to prevent sporadic viremic episodes.

A prior study showed that HIV controllers had higher T cell activation than seronegative donors [10]. There are additional studies that have shown the presence of immune activation in EC [29, 53]. However our data shows that excess T cell activation is only present in the EC^{lo} group and not among the EC^{hi} individuals. This was not influenced by racial composition differences between the 2 EC groups since similar levels of immune activation and exhaustion markers were noted between Caucasians and African-Americans (EC^{lo} and NC); data not shown. One likely explanation for the discrepancy between our EC work and those by other groups could be that prior studies were perhaps largely based upon EC patients with low CD4%.

Evaluation of numerous immune indices showed that the EC^{hi} group demonstrates several hallmark characteristics of preserved immune integrity. Notably, these EC^{hi} individuals maintained a higher CD4% that was associated with lower expression of multiple markers of activation and exhaustion. Additionally, in this group, maintenance of CD28 expression, a co-stimulatory molecule which is commonly reduced during chronic HIV infection was retained. A recent study found that CD73, a coactivator of T cells [13] was elevated in all EC. However our data shows that this is only observed for the EC^{hi} group and therefore cumulatively indicates that the EC^{hi} group manifests a phenotype of functionally responsive T cells that is similar to healthy seronegative adults.

Immune activation has been associated with reduced CD4 T cell recovery. Although we did not see differences in the sCD14 between the two EC groups in our limited sample size, it is likely that EC^{hi} have lower levels of products of microbial translocation, due to less severe CD4 depletion early in infection and lower activation induced cell death, both of which could contribute to the CD4% preservation in this group. In contrast, the EC^{lo} behave more like the immunologic non-responders on ART[54] who have been shown to have increased microbial translocation and immune activation which may drive CD4 depletion and hence the failure to normalize CD4 T cell counts. Interestingly in patients with idiopathic CD4 lymphocytopenia (ICL), increased levels of LPS were observed highlighting a potential association between translocation of microbial products, immune activation and altered CD4 T cell homeostasis [55].

Recent studies have highlighted the usefulness of CD4/CD8 ratio for gauging immune restoration [47, 56]. In our study, we observed a strong correlation between CD4% and the CD4/CD8 ratio among elite controller individuals ($r=0.84$; $p\text{-value} < 0.0001$). Furthermore, the median CD4/CD8 ratio for the EC^{hi} mirrored the HIV seronegatives whereas the EC^{lo} and the HIV non controllers exhibited a similar ratio. Additionally, significant correlations were observed when either the CD4% ($r = -0.79$, $p=0.0001$) or the CD4/CD8 ratio ($r = -0.80$, $p < 0.0001$) was compared with % CD8 T cell immune activation (HLADR+CD38+).

In summary, our data suggest that a subgroup of EC with preserved CD4 T cell percentages may be immunologically normal and would not derive clinical benefit from ART. In contrast, EC with evidence of CD4 depletion may well benefit from the implementation of ART to suppress low level viremia and reduce immune activation and exhaustion. While our findings based on a small sample size cannot be used to dictate treatment choices, these data can inform the design of future studies focused on elite controllers and on immunomodulatory therapies that are aimed at improving the immune function in chronic HIV infection. Our findings further support CD4% as an important marker in subdividing EC to better evaluate results from future studies designed to determine the need for ART in this group of individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

HIV-1	human immunodeficiency virus type 1
EC	elite controllers
SN	seronegatives and NC, non-controllers

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Figure 1. Longitudinal clinical data for all elite controller individuals

CD4 values (% and absolute count) for all patients in each of the two elite controller groups (EC^{hi} and EC^{lo}) for each longitudinal time point were averaged and a linear regression line was fit to each data set. The slopes of the regression lines were compared using two-tailed ANCOVA and the p-value is shown (A and B). The change between the first and last clinical value was calculated for each person and the median change between the 2 groups was compared with two-tailed Mann–Whitney test (C and D).

Figure 2. Surface expression of immune activation markers on T cells and its correlation with CD4%

CD8 (A) and CD4 (B) T cells were surface stained for the single/dual expression of activation markers CD38 and HLA-DR. Bars represent median values $\pm$ interquartile range, groups were compared by two-tailed Mann-Whitney test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). The association between CD4% and co-expression of immune activation markers on CD8 (C) and CD4 (D) T cells was determined using two-tailed Spearman nonparametric correlation.

Figure 3. Surface expression of markers of T cell exhaustion

CD8 (A and C) and CD4 (B and D) T cells were surface stained for the exhaustion markers PD-1, CD160 and TIM3. Expression of PD-1 and CD160 (A & B) as well as TIM-3, and coexpression of CD160⁺PD-1⁺ (C & D) are shown. Bars represent median values +/- interquartile range, groups were compared by two-tailed Mann-Whitney test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Figure 4. Surface expression of markers of co-stimulation on CD8 T cells and its correlation with immune activation

(A) CD8 T cells were surface stained for the immunosenescence marker CD57 and the co-stimulatory molecules CD28 and CD73. Bars represent median values $\pm$ interquartile range, groups were compared by two-tailed Mann-Whitney test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Correlation between % activated CD8 T cells and CD73 expression on cells from all elite controllers (B), seronegatives (C), and non-controllers (D) was determined by two-tailed Spearman nonparametric correlation.

Table 1

Demographic and clinical information of the study cohort.

Category ^a	EC ^{hi} (n=9)	EC ^{lo} (n=7)	NC (n=9)	SN (n=8)	P-value ^b	P-value (EC ^{hi} vs. EC ^{lo}) ^c
Sex:						
Female	56	43	44	50	1.00	1.00
Male	44	57	56	50		
Race:						
African-American	100	57	56	50	0.06	0.06
Caucasian	0	43	44	50		
Age:						
	48	54	44	44	0.008	0.25
HIV Risk Factors:						
Heterosexual	67	43	56	NA		
MSM	22	57	33	NA	0.6	0.19
IVDU	11	0	0	NA		
Unknown	0	0	11	100		
CD4^a						
Count	1158	698.5	298	NA	0.0003	0.0285
%	52	27.5	14	NA	<0.0001	0.0002
Nadir	895	449.5	147	NA	0.0001	0.0079
Viral Load^a						
	19	59.5	146668	NA	0.0001	NA
Co-Infections:						
HCV	11	0	11	NA	1.00	1.00
HBV	11	14	11	NA	1.00	1.00
HLA-I:						
B*27	0	14	0	12.5	0.34	0.44
B*57	33	43	33	0	0.22	0.63
B*58	33	0	11	25	0.45	0.21

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^a One patient in each of the EC^{hi} and EC^{lo} groups was followed longitudinally and data from both time points are included. Categorical and continuous variables are expressed as percentage and median values respectively. Groups being compared are elite controllers with median longitudinal %CD4 > 40 (EC^{hi}), elite controllers with median longitudinal %CD4 < 40 (EC^{lo}), non-controllers (NC), and seronegatives (SN).

^{b,c} Categorical variables were compared using Fisher's exact test and continuous variables were compared using non-parametric Kruskal-Wallis test to compare all groups and Mann-Whitney test to compare EC^{hi} and EC^{lo} groups.