Normal T-cell activation in elite controllers with preserved CD4$^+$ T-cell counts

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Background: HIV elite controllers suppress HIV viremia without antiretroviral therapy (ART), yet previous studies demonstrated that elite controllers maintain an activated T-cell phenotype. Chronic immune activation has detrimental consequences and thus ART has been advocated for all elite controllers. However, elite controllers are 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 elite controllers needing ART.

Methods: Sixteen elite controllers were divided into two groups based on CD4% (EC^{hi} $>$ 40% and EC^{lo} $\leq$40%), and T-cell subsets were analyzed for markers of memory/differentiation (CD45RA, CCR7, CD28), activation (CD38/HLA-DR), immunosenescence (CD57), costimulation (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 with the EC^{lo} group and similar to the seronegative controls. The EC^{hi} group expressed higher levels of costimulatory 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 6 years.

Conclusion: Elite controllers with preserved CD4$^+$T cells (EC^{hi}) have normal T-cell and monocyte phenotypes and therefore may have limited benefit from ART. CD4% can be an important marker for evaluating future studies aimed at determining the need for ART in this group of individuals.

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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, represent an excellent in-vivo model of spontaneous HIV control [1,2].

Published data suggest that elite controllers maintain durable viral control through an armamentarium of virological, immunological and genetic features [1,3,4].

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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], upregulation of survival factors such as bcl-2 [11], upregulation of cyclin-dependent kinase inhibitors such as p21 [12] and higher frequency of expression of costimulatory molecule CD73 [13]. Furthermore, cells from elite controllers are less susceptible to infection and effectively suppress *in-vitro* HIV replication [8,14]. The elite controllers 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, elite controllers fail to clear HIV infection. Ultra-sensitive assays measuring pVL indicate that residual pVL remains higher in some elite controllers than in ART-treated individuals [17,18]. Additionally, $CD4^+$ cells from elite controllers can harbor pathogenic replication competent virus that may contribute to excess T-cell activation [19,20]. Interestingly a recent simian immunodeficiency virus 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 elite controllers with increased inflammatory monocytes compared with HIV-uninfected controls [22]. Recently, elite controllers have also been shown to have elevated levels of key soluble inflammatory markers [23] and higher hospitalization rates than ART-treated individuals [24].

Upregulation 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 elite controllers 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 comorbidities [10,28,29].

Since persistent immune activation and the ensuing inflammation can have detrimental consequences, it has thus been suggested all elite controllers may benefit from ART [19,30]. However, HIV controllers are not a homogenous group [31–34] whereby some individuals maintain absolute $CD4^+$ cell 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 elite controllers 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. Elite controllers with preserved $CD4^+$ T-cell percentages (EC^{hi}) had significantly lower levels of activated/exhausted HIV-specific $CD8^+$ T cells than elite controllers 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 whereas no evidence was obtained to suggest that such treatments would benefit the EC^{hi} group.

Materials and methods

Study cohort

Cryopreserved peripheral blood mononuclear cells (PBMC) and plasma samples from 25 HIV-1-infected patients receiving care at the 1917 clinic at the University of Alabama at Birmingham and eight HIV-1 seronegative volunteers enrolled in the Alabama Vaccine Research Clinic at University of Alabama at Birmingham (UAB) were used in this study. All HIV-infected patients were off ART. The elite controllers 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 elite controllers 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 two comparator groups, that is HIV seronegative and HIV-1 noncontrollers (pVL $> 10\,000$ copies/ml, $n = 9$). Local institutional review board 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, Life Technologies, Carlsbad, California, USA), washed, and surface labeled with the

following antibodies: *Panel 1* – anti-CD3 (APC-eFluor 780, eBioscience, San Diego, California, USA), anti-CD4 (Qdot655, Invitrogen), anti-CD8 (V500, BD (Becton Dickinson, Franklin Lakes, New Jersey, USA)), anti-CD45Ra (Brilliant Violet 421, BD), anti-CCR7 (PercpCy5.5, 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 (PercpCy5.5, BD), anti-CD73 (PECy7, BD), anti-PD1, anti-CD160 (Alexa488, BD), and anti-TIM3 (Brilliant Violet 421, Biolegend, San Diego, California, USA); *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 min, followed by washing and staining for all other surface markers for 20 min at 4°C. Samples were washed, fixed in 2% paraformaldehyde and analyzed on a BD flow cytometer (LSR II). At least 1 00 000 $CD3^+$ events were acquired and the data were analyzed using FlowJo software (version 9.7, Tree Star Inc., Ashland, Oregon, USA). Frequency and mean fluorescence intensity data were analyzed and compared for all single markers. Boolean combination gating was performed to analyze combinations of three or more markers.

Soluble CD14 ELISA

Plasma levels of sCD14 were quantified using a Human sCD14 Quantikine ELISA kit (R&D Systems, Minneapolis, Minnesota, USA). Briefly, plasma samples were prefiltered, UV inactivated, diluted 1 : 200 and incubated in plates precoated with antihuman sCD14 antibody for 3 h. After washing, plate-bound sCD14 was detected with an horseradish peroxidase-conjugated antisCD14 antibody for 1 h. The plate was developed using tetramethylbenzidine for 30 min before 2N H_2SO_4 stop solution was added. Plate-bound sCD14 was quantified by measuring the absorbance at 450 nm with a wavelength correction at 540 nm 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 nonparametric Kruskal–Wallis test was used to assess differences among the four groups. The expression of markers between the groups was compared pairwise using the nonparametric Mann–Whitney test and covariation between two variables was quantified using the nonparametric Spearman correlation.

Clinical data (CD4% and absolute $CD4^+$ cell 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 nonparametric Mann–Whitney test. All analyses were performed using Prism software (version 6, GraphPad Software Inc., La Jolla, California, USA).

Results

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

We categorized two elite controllers 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 elite controller 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 elite controllers groups (Fig. 1) with respect to absolute $CD4^+$ cell counts and CD4%. Although 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% as expected (Fig. 1a and b). Although most individuals demonstrated relatively stable CD4% and absolute count over time (Fig. 1c and d), there was a trend for a decrease in CD4% (Fig. 1c, $P = 0.067$) in the EC^{lo} group compared with the EC^{hi} group.

Furthermore, pVL was lower in the EC^{hi} group and with fewer intermittent viral blips compared with the EC^{lo} group (supplementary Figure 1, http://links.lww.com/QAD/A768). Given these clinical distinctions among the two elite controllers 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 paticipents in each group are shown in Table 1. Most HIV-infected volunteers 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 elite controllers groups. Detailed demographic and clinical characteristics for each study participant are shown in supplementary Table 1, http://links.lww.com/QAD/A768. One individual in each group (participant 9 and 16) was evaluated for immunogenicity at two different time points (Table 1 and supplementary Table 1, http://links.lww.com/QAD/A768).

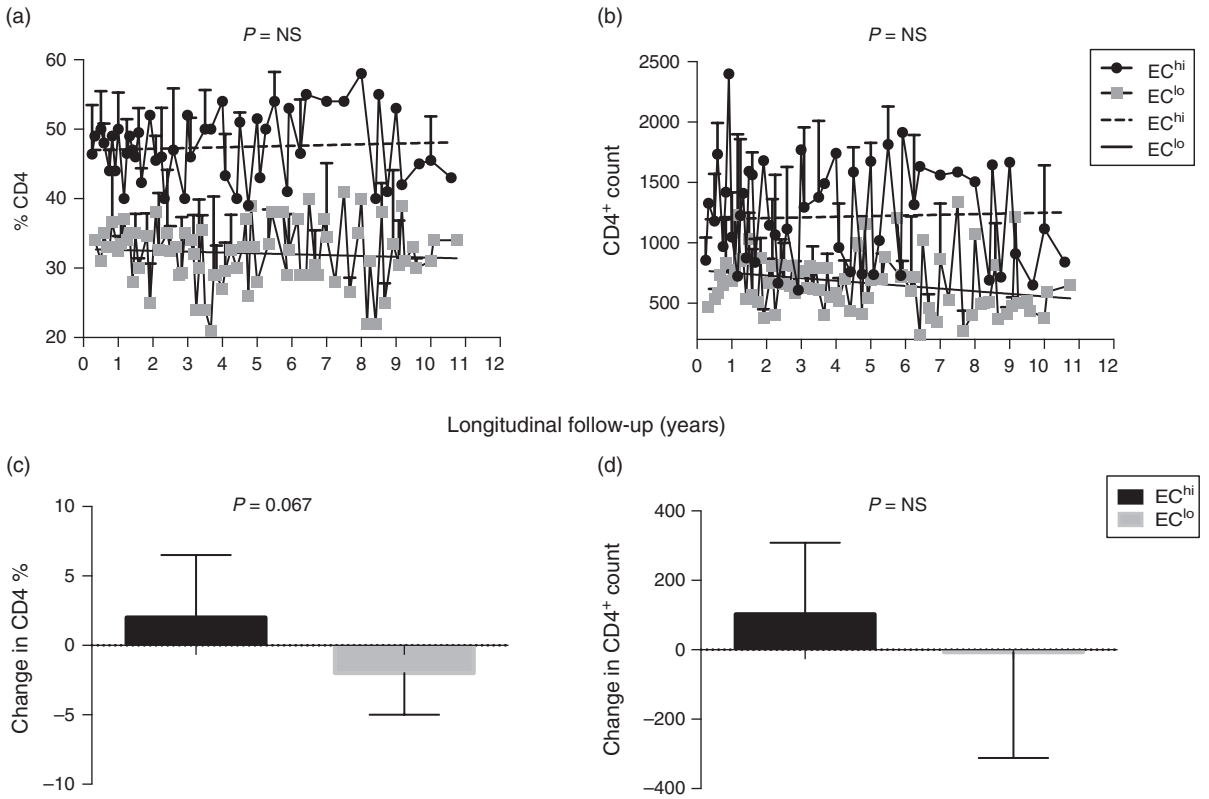

Fig. 1. Longitudinal clinical data for all elite controller individuals. $CD4^+$ values (% and absolute count) for all patients in each of the two elite controller groups (EC^{hi} and EC^{lo}) for each longitudinal time point were averaged and a linear regression line was fit to each data set. The slopes 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 two groups was compared with two-tailed Mann–Whitney test (c and d).

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 four groups of participants. A comparative analysis of the elite controllers 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 with the EC^{lo} group (Fig. 2a). Similar data were also observed in the $CD4^+$ T cells (Fig. 2b). Additionally, for CD8 T cells, a trend for lower expression of $CD38^+$ for the EC^{hi} was observed (23 vs. 38%, $P = 0.058$; Fig. 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 elite controllers groups were compared with the seronegative and noncontrollers (supplementary Tables 2 and 3, http://links.lww.com/QAD/A768).

An inverse correlation was previously reported between absolute $CD4^+$ cell counts and immune activation of T cells [10]. Similarly, in our elite controllers cohort, negative correlations were observed between CD4% and $CD38^+$ HLA-DR^+ expression on $CD8^+$ T cells ($r = -0.8306$, $P < 0.0001$; Fig. 2c) and $CD4^+$ T cells ($r = -0.7742$, $P = 0.0002$; Fig. 2d). Correlations between $CD8^+$ T-cell activation and CD4% were not observed for the noncontrollers 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 naive ($CCR7^+$ $CD28^+$ $CD45RA^+$), 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, seronegative, EC^{hi}, and EC^{lo} had significantly higher frequency of central memory T cells than noncontrollers ($P = 0.0002$; $P = 0.036$; $P = 0.050$ 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

Table 1. Demographic and clinical information of the study cohort.

Category[a]	EC^{hi} ($n=9$)	EC^{lo} ($n=7$)	Noncontrollers ($n=9$)	Seronegative ($n=8$)	*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
Whites	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
Coinfections						
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

HBV, hepatitis B virus; HCV, hepatitis C virus; HLA, human leukocyte antigen; IVDU,intravenous drug user.
[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}), noncontrollers, and seronegatives.
[b]For all groups, categorical variables were compared using Fischer's exact test and continuous variables were compared using the non-parametric Kruskal–Wallis test.
[c]Mann–Whitney test was used to compare EC^{hi} and EC^{lo} groups.

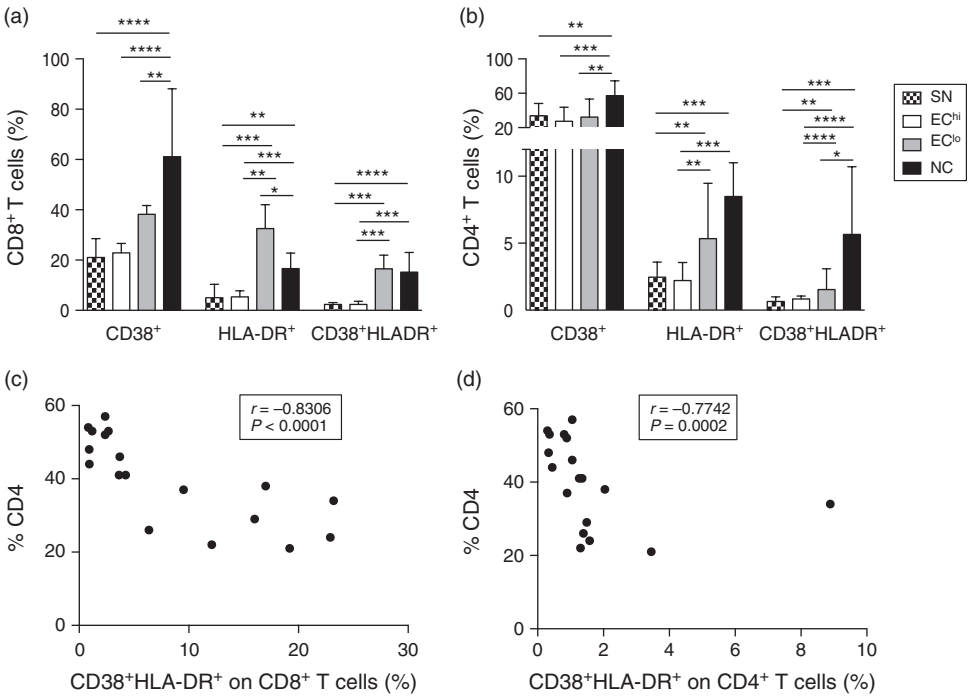

Fig. 2. Surface expression of immune activation markers on T cells and its correlation with CD4%. $CD8^+$ (a) and $CD4^+$ (b) T cells were surface stained for the single/dual expression of activation markers CD38 and HLA-DR. Bars represent median values ± interquartile range, groups were compared by two-tailed Mann–Whitney test ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$). The association $CD4^+$ between CD4% and coexpression of immune activation activation markers on $CD8^+$ (c) and $CD4^+$ (d) T cells was determined using two-tailed Spearman nonparametric correlation.

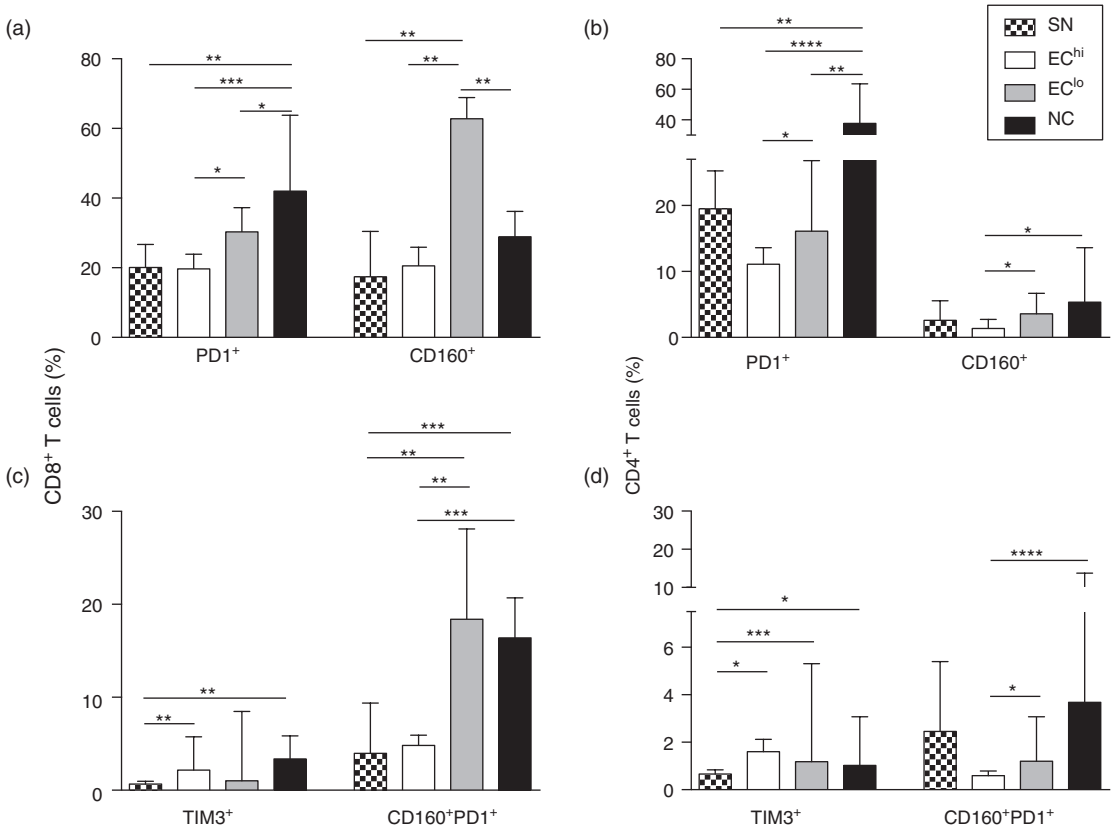

Fig. 3. Surface expression of markers of T-cell exhaustion. $CD8^+$ (a and c) and $CD4^+$ (b and d) T cells were surface stained for the exhaustion markers PD-1, CD160 and TIM3. Expression of PD-1 and CD160 (a and b) as well as TIM-3, and coexpression of $CD160^+PD\text{-}1^+$ (c and d) are shown. Bars represent median values ± interquartile range, groups were compared by two-tailed Mann–Whitney test ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$).

with the EC^{lo} group ($P=0.03$, supplementary Figure 2, http://links.lww.com/QAD/A768). Additionally, the EC^{hi} group had higher frequency of naive $CD8^+$ T cells compared with 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 PD-1 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, that is PD-1, CD160 and TIM-3 [45]. The EC^{hi} had decreased $PD\text{-}1^+$ (20 vs. 30%, $P=0.0418$), $CD160^+$ (21 vs. 63%, $P=0.003$) and $CD160^+$, $PD\text{-}1^+$ (5 vs. 17%, $P=0.003$) compared with EC^{lo} on the $CD8^+$ T cells (Fig. 3a and c). A similar pattern of $PD1^+$ and $CD160^+$ expression was also seen in $CD4^+$ T cells ($P=0.006$) (Fig. 3b and d). The EC^{hi} also had lower mean fluorescence intensity of $PD\text{-}1^+$ on $CD8^+$ (4297 vs. 4854, $P=0.006$) and $CD4^+$ T cells (4641 vs. 5041, $P=0.04$) compared with EC^{lo}. Details of other marker combinations that were different between all groups are shown in supplementary Tables 2 and 3, http://links.lww.com/QAD/A768.

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} (Fig. 4a). In each case, EC^{hi} were similar to seronegatives, whereas the EC^{lo} were comparable to noncontrollers. Additional data for the inter-group comparisons is shown in supplementary Tables 2 and 3, http://links.lww.com/QAD/A768.

A prior publication showed an inverse correlation between $CD73^+$ expression and $CD8^+$ T-cell activation [13]. We too observed a negative correlation for the

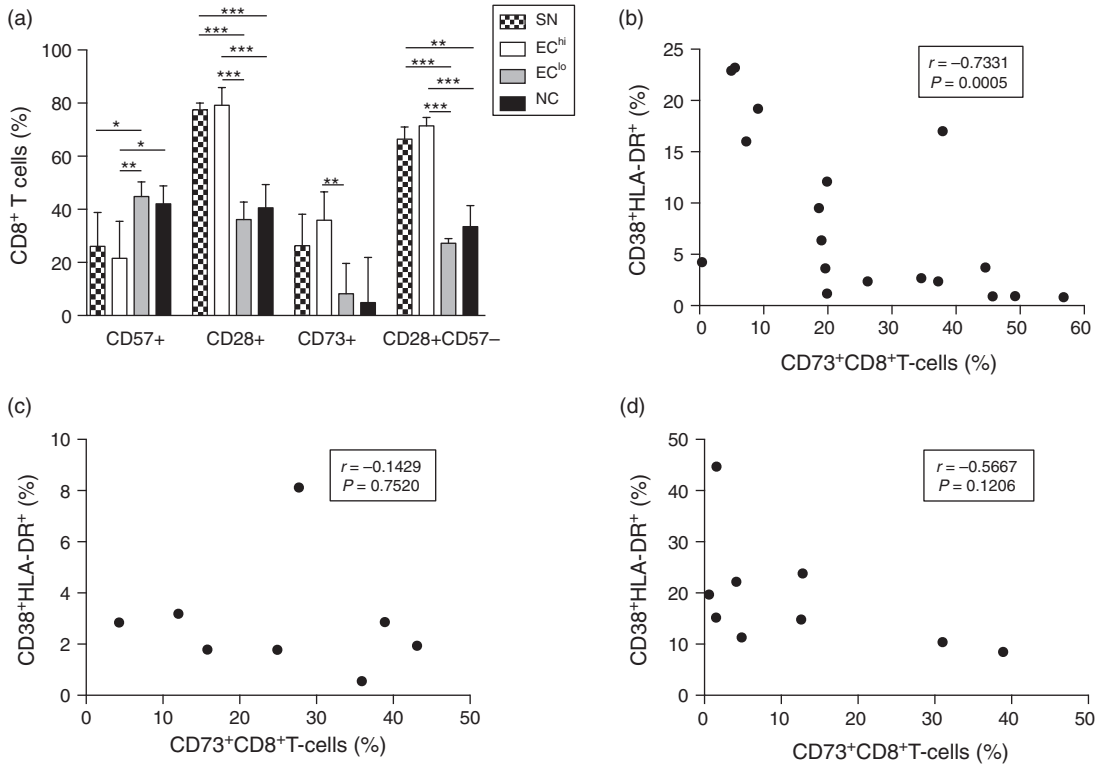

Fig. 4. Surface expression of markers of costimulation on CD8$^+$ T cells and its correlation with immune activation. (a) CD8$^+$ T cells were surface stained for the immunosenescence marker CD57 and the costimulatory molecules CD28 and CD73. Bars represent median values ± interquartile range, groups 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 noncontrollers (d) was determined by two-tailed Spearman nonparametric correlation.

combined elite controllers group which was most likely driven by the EC^{hi} group (Fig. 4b). However, we did not see a correlation for either the seronegative or noncontrollers although there was a trend in the latter group (Fig. 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 nonclassical / patrolling (CD14$^+$ CD16^{++}) as previously described [49]. The EC^{hi} subgroup showed decreased frequency of intermediate / inflammatory monocytes compared with the EC^{lo} (median 1 vs. 2.2%, $P = 0.001$, supplementary Figure 3, http://links.lww.com/QAD/A768). This inflammatory subset also expressed lower median levels of HLA-DR when compared with 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 elite controllers groups – supplementary Figure 3, http://links.lww.com/QAD/A768. Plasma levels of sCD14 were not different among the two elite controllers groups or the elite controllers overall group compared with seronegative and noncontrollers.

Discussion

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 elite controllers also have evidence of immune activation and may benefit from ART as well. Such prior studies have generally considered elite controllers 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 elite controllers cohort, we have shown that CD4% is an easily quantifiable benchmark for gauging the clinical heterogeneity in the elite controllers group as reflected by the stark differences in the immune

phenotypes observed. Specifically, our data demonstrated that $CD8^+$ T cells from elite controllers 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 with HIV-infected patients including elite controllers with low CD4% (EC^{lo}) and noncontrollers. Future studies will assess the clinical outcome from ART in elite controllers with low CD4%.

Some of the heterogeneity in elite controllers may stem from the varied clinical definitions that have been used to identify elite controllers [51]. As recommended in a recent review of elite controllers literature, we used the criteria of elite controllers 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 elite controllers 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 elite controllers [29,53]. However our data show 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 two elite controllers groups since similar levels of immune activation and exhaustion markers were noted between whites and African-Americans (EC^{lo} and noncontrollers); data not shown. One likely explanation for the discrepancy between our elite controllers work and those by other groups could be that prior studies were perhaps largely based upon elite controllers 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 costimulatory 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 elite controllers. However our data show 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 elite controllers 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 nonresponders 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 value $= <0.0001$). Furthermore, the median $CD4^+/CD8^+$ ratio for the EC^{hi} mirrored the HIV seronegatives whereas the EC^{lo} and the HIV noncontrollers 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 elite controllers with preserved $CD4^+$ T-cell percentages may be immunogically normal and would not derive clinical benefit from ART. In contrast, elite controllers with evidence of $CD4^+$ depletion may well benefit from the implementation of ART to suppress low-level viremia and reduce immune activation and exhaustion. Although 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 elite controllers to better evaluate results from future studies designed to determine the need for ART in this group of individuals.

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Conflicts of interest

There are no conflicts of interest.

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