Published in final edited form as:

J Clin Immunol. 2011 October ; 31(5): 873–881. doi:10.1007/s10875-011-9550-6.

Baseline Immune Phenotypes and CD4+ T Lymphocyte Responses to Antiretroviral Therapy in Younger versus Older HIV-infected Individuals

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Abstract

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Potential conflicts of interest Judith Currier has received research grant support to UCLA for clinical trials from Merck and Company, Tibotec Therapeutics, and Schering Plough in the past 48 months. In addition, she is involved in clinical trials in which drugs have been donated to NIH from Tibotec, Merck, Gilead, Abbott, Glaxo Smith Kline, and Bristol Myers Squibb. Ronald Bosch has served on the advisory board of Tibotec. Risa Hoffman, Beth Jamieson, Christina Kitchen, Ingrid Schmid, Yuda Zhu, Kara Bennett, Ronald Mitsuyasu have no conflicts of interest.

Objective—The purpose of the study was to determine associations between pre-antiretroviral therapy (ART) senescent CD8+ T lymphocytes and naïve versus non-naïve CD8+ and CD4+ T lymphocyte subpopulations and CD4+ responses after initiation of ART in younger versus older individuals.

Methods—Retrospective analysis of 100 subjects with pre-ART cryopreserved peripheral blood mononuclear cells samples was performed with flow cytometry. Subjects were divided into four groups by age (30–50 years or >50 years) and 96-week CD4+ response (<100 or >200 cells/mm³). All subjects had 96-week viral suppression to <50 copies/ml. Regression was utilized to investigate associations between pre-ART CD8+ and CD4+ T cell phenotypes with age and CD4+ response categories.

Results—Individuals <50 years had a lower frequency of senescent CD8+ T lymphocytes of the CD56+57+, CD56+, and CD28– phenotypes (95% CI –3.6 to –0.02; 95% CI –4.2 to –0.03; 95% CI –12.5 to –1.4, respectively) and a higher frequency of naïve (CD45RA+CD28+) CD8+ T lymphocytes (95% CI 2.6 to 10.9). Younger age and good CD4+ response were associated with a higher frequency of pre-ART naïve CD4+ T cells (95% CI 2.0 to 16.4 and 95% CI 1.5 to 15.6, respectively).

Conclusions—Prior to ART, younger HIV-infected individuals have a higher frequency of naïve CD4+ and CD8+ T cells and lower frequency of senescent CD8+ T cell phenotypes.

Keywords

HIV/AIDS; aging; immune risk phenotype; antiretroviral therapy; immune senescence

Introduction

Epidemiologic data suggest HIV-infected individuals over 50 years have slower immunological responses to potent antiretroviral therapy (ART) despite sustained virologic responses [1–3]. At least one study found a significant association between age older than 50 years at ART initiation, low CD4+ T lymphocyte response at 12 months, and increased all-cause mortality, with an adjusted hazard ratio for mortality of 4.26 (95% CI, 1.68–10.83) [4]. Additional research suggests older HIV-infected adults have poorer outcomes compared with younger patients, including more rapid progression to AIDS, and increased risk of death from both AIDS-related and non-AIDS causes including cardiovascular disease, renal disease, liver disease, and malignancies. These findings persist after adjusting for CD4+ T lymphocyte response, suggesting that age contributes independently to clinical progression of HIV [5, 6].

Immune senescence has emerged as the term used to describe age-related functional and phenotypic alterations in T lymphocytes, including the accumulation of terminally differentiated effector cells, reduced numbers of naïve CD8+ and CD4+ T cells, reduced T lymphocyte proliferation, and increased production of inflammatory cytokines [7, 8]. Immune senescence is thought to be driven by chronic antigenic stimulation, leading to expansion of poorly functional CD28– CD8+ T lymphocytes [9–11]. These changes result in chronic inflammation, subclinical infections, reduced response to vaccines, and increased morbidity and mortality [12]. Longitudinal studies in HIV-negative elderly populations have identified an immune risk phenotype (IRP) that is associated with increased mortality and defined by inverted CD4+/CD8+ ratio, persistent CMV infection, and an increase in senescent CD8+ T lymphocytes with the following phenotypes: CD28–, CD27–, CD56+, CD57+, CD45RA+, CD45RA+CD45RO+ [13–16]. Avoidance of this specific immune senescent phenotype has been found to be protective for survival to 100 years of age in very elderly patients [17]. At least one additional study has shown that the combination of this

IRP plus elevated IL-6 and C-reactive protein is predictive of mortality in the elderly, independent of cardiovascular disease, Alzheimer's dementia, and diabetes mellitus [12].

Data from the HIV/AIDS literature suggest that individuals with chronic HIV infection develop accelerated aging of T lymphocytes, resulting in complete and permanent loss of CD28, loss of important cytokine signaling pathways, and T cell activation, as well as a reduced T cell lifespan [18–20]. In addition, patients with rapid HIV progression have been found to have an accelerated reduction of the CD28 cell surface expression level among CD8+ and CD4+ T lymphocytes [21]. We sought to characterize pre-ART baseline CD8+ and CD4+ T lymphocyte phenotypes in HIV-infected individuals in younger (35–50 years) and older (>50 years) individuals with viral suppression to <50 copies/ml on ART who experienced a poor (<100 cells/mm³) or good (>200 cells/mm³) CD4+ T lymphocyte increase 96 weeks after the initiation of potent ART.

Methods

Subject Source and Selection

The AIDS Clinical Trials Group (ACTG) Longitudinal Linked Randomized Trials (ALLRT) cohort (A5001) comprises HIV-infected individuals enrolled in randomized ACTG antiretroviral therapy studies who agree to be followed longitudinally as part of an additional observational study that continues beyond the end of the parent ACTG protocol in which they are enrolled. Standardized demographic data, clinical information, and laboratory parameters are collected at baseline and then every 16 weeks indefinitely.

Initially, 158 subjects were selected from ACTG studies 384, 388, A5014, A5095, A5142, and A5001 based on age and CD4+ T lymphocyte response after 96 weeks of potent ART. All patients were ART-naïve at baseline, and 84% were initiated on therapy within 24 h of obtaining baseline blood samples (mean duration from baseline blood collection to ART initiation 0.5 days, range 0–26 days). Patient selection required viral suppression on ART to <400 copies/ml by week 16 and at every subsequent 16-week follow-up, as well as a viral load of <50 copies/ml at the 96-week (or 80-week if no 96-week) evaluation. Subjects were selected regardless of ART regimen. Co-variables provided from the database included age, race, ART regimen (PI-based, NNRTI-based, NRTI only), sex, current or previous intravenous drug use, hepatitis B surface antigen and core antibody status, and hepatitis C antibody. Exemption for the study was granted by the UCLA Internal Review Board.

Younger age was defined as 30–50 years, and older age was defined as >50 years of age, at baseline. The age range for younger individuals was selected based on availability of sufficient numbers of patients in this cohort to make comparisons with the >50 year population. CD4+ T lymphocyte response to therapy was defined as a CD4+ T cell gain of >200 cells/mm³ and nonresponse as a CD4+ T cell gain of <100 cells/mm³ between baseline (pre-ART) and 96-week follow-up. The CD4+ T lymphocyte count utilized to categorize individuals as responders versus nonresponders was an average of the 80- and 96-week CD4+ T lymphocyte values.

Because of the low number of >50-year-old subjects in the nonresponder category with available peripheral blood mononuclear cells (PBMC), flow cytometry was performed on all baseline pre-ART samples in this group ($n=23$). After removing those with insufficient PBMC numbers to perform the flow analyses, we randomly selected 45 baseline pre-ART samples from the pool of available 30–50-year-old subjects in both the responder and nonresponder categories. For the group of >50-year-old responders, we had exactly 45 subjects with sufficient PBMC availability. Of the 158 samples shipped to UCLA for analysis, 47 had to be excluded because no viable cells could be recovered after thawing as

determined by flow cytometric assessment of membrane integrity by DNA staining. We then further excluded subjects with cell viability less than 10%, leaving a total of 100 samples, divided as follows: older nonresponders ($n=12$), younger nonresponders ($n=31$), older responders ($n=26$), younger responders ($n=31$).

Flow Cytometry

Cryopreserved PBMCs were thawed and stained with the following antibody panel: (1) Tube 1: IgG1 FITC/IgG1 PE/CD3 PerCP/CD4 PE-Cy7/IgG1 APC/CD8 APC-Cy7; (2) Tube 2: CD45RA FITC/CD28 PE/CD3 PerCP/CD4 PE-Cy7/CD45RO APC/CD8 APC-Cy7; (3) Tube 3: CD57 FITC/CD28 PE/CD3 PerCP/CD4 PE-Cy7/CD56 APC/CD8 APC-Cy7.

The DNA dye DAPI was added to each tube for dead cell exclusion. Samples were analyzed on a BD LSRII flow cytometer (BD Biosciences) equipped with a 488-nm blue laser, a 640-nm red laser, and a 407-nm violet laser and the appropriate filter sets for the fluorochromes used in the staining panel. A minimum of 10,000 CD4⁺ and CD8⁺ T lymphocytes were collected whenever possible. Data analysis was performed with FACSDiva software (BD Biosciences).

Statistical Methods

Analysis of variance (ANOVA) and Fisher's exact tests were used to evaluate differences in baseline characteristics among each of the four groups (younger responders, younger nonresponders, older responders, older nonresponders). Comparisons were also made between characteristics of those subjects who were included versus excluded in the analysis using the 10% viability cut-off. The association of CD8⁺ and CD4⁺ T cell flow markers with age (30–50 years and >50 years) and CD4⁺ T lymphocyte response status (responder >200 cells/mm³ and nonresponder <100 cells/mm³) at 96-week follow-up was modeled with linear regression. All 95% confidence intervals were adjusted and calculated as the difference from the referent. Multivariate regression was performed for all univariate results with P values less <0.15. Co-variables for this analysis included race, ART regimen (NNRTI versus PI), hepatitis B co-infection (defined by positive hepatitis B surface antigen), and hepatitis C co-infection (defined by positive hepatitis C virus antibody).

Our study was not formally powered to show a specific difference in immune senescent T lymphocyte subsets between the four groups, as there are no data regarding the frequency of these subpopulations in HIV-infected individuals on which to base sample size calculations. Therefore, we performed a pilot study to obtain preliminary data about baseline CD8⁺ and CD4⁺ T lymphocyte subpopulations and CD4⁺ T cell responses after initiation of ART in older compared with younger individuals. We hypothesized that older individuals with poor CD4⁺ T cell responses would have the highest rates of senescent marker expression and that those individuals with features of the IRP would be less likely to have a good immunologic response to ART, independent of age.

Results

Baseline Subject Characteristics

For the 100 subjects included in the final analysis, baseline characteristics of the four groups are summarized in Table I. Due to pre-selection of groups by age and CD4⁺ T lymphocyte response, significant differences were found for age ($P<0.001$) and CD4⁺ T cell change from baseline to 96 weeks ($P<0.001$). There were no significant differences noted in the proportion of women, intravenous drug use, hepatitis B and C antibody prevalence, non-white subjects, or individuals on protease inhibitor-containing ART. Furthermore, there

were no significant differences in baseline viral load ($\log_{10}$ RNA) among the four pre-selected groups.

Characteristics of Subjects Included versus Excluded from the Analysis Using the 10% Viability Cut-Off

Characteristics of individuals included versus excluded using the 10% viability cut-off were evaluated. There was a non-significant trend toward older individuals being excluded for low viability. There were no significant differences in mean baseline CD4+ T cell counts, mean CD4+ T lymphocyte change at 96-week follow-up, or mean baseline $\log_{10}$ RNA (Table II).

Regression Analysis: Association of CD8+ T cell Subsets with Age and CD4+ T Lymphocyte Response to Antiretroviral Therapy

Linear regression was performed to simultaneously examine the associations between CD8+ T lymphocyte markers of interest and age (younger versus older) and response category (responder versus nonresponder; Table III). Younger age was associated with a lower proportion of the senescent CD8+ T cell phenotypes CD56+57+ and CD56+, with an average of 1.8 fewer percentage points of CD56+ 57+ T lymphocytes and 2.1 fewer percentage points of CD56+ T lymphocytes compared with older individuals (95%CI -3.6 to -0.02, $P=0.047$ and 95%CI -4.2 to -0.03, $P=0.05$, respectively). Younger individuals also had a lower proportion of senescent CD28- CD8+T lymphocytes, with an average of 6.9 fewer percentage points compared with older individuals (95%CI -12.5 to -1.4, $P=0.02$). Unexpectedly, CD4+ T cell responders had a higher proportion of CD8+ T lymphocyte senescent markers CD56+57+ and CD56+, with an average of 2.1 more percentage points of CD56+57+ T lymphocytes and 2.2 more percentage points of CD56+ T lymphocytes compared with individuals with CD4+ T cell nonresponse (95%CI 0.31 to 3.8, $P=0.02$ and 95%CI 0.17 to 4.3, $P=0.03$, respectively).

We also evaluated associations between naïve versus non-naïve CD8+ T lymphocytes with age and CD4+ T cell response categories. Younger individuals had a greater proportion of naïve CD8+ T lymphocytes (CD45RA+CD28+) with an average of 6.8 more percentage points compared with older individuals (95%CI 2.6 to 10.9, $P=0.002$).

In a multivariate analysis that included race, intravenous drug use, ART regimen, and hepatitis B and C antibody positivity, T lymphocyte marker associations that were significant in the analysis with only age and response category remained significant with all these co-variates in the model (all P values <0.05).

Regression Analysis: Association of CD4+ T Cell Subsets with CD4+ T Lymphocyte Response to Antiretroviral Therapy

Regression analyses similarly evaluated pre-ART naïve CD4+ T cell frequencies for association with age category and CD4+ T cell response to ART (Table III). Younger age and CD4+ T lymphocyte response were associated with a higher frequency of pre-ART naïve CD4+ T cells (CD45RA +CD28+), with younger individuals having 9.2 more percentage points and CD4+ T lymphocyte responders 8.5 more percentage points of naïve CD4+ T lymphocytes (95%CI 2.0 to 16.4, $P=0.01$ and 95%CI 1.5 to 15.6, $P=0.02$). In contrast, younger individuals and CD4+ T cell responders had a lower frequency of pre-ART central memory CD4+ T lymphocytes (CD45RA-CD28+), with an average of 7.9 and 14.0 fewer percentage points compared with older individuals and CD4+ T cell nonresponders (95% CI -15.6 to -0.17, $P=0.045$ and 95%CI -21.5 to -6.4, $P<0.001$). Of note, senescent CD28- CD4+ T lymphocytes were not significantly different by age or CD4+ T cell response category.

Results for the naïve CD4+ phenotype (CD45RA+CD28+) retained significance at the $P<0.05$ level in a multivariate analysis that additionally included race, intravenous drug use, ART regimen, and hepatitis B and C antibody positivity. For the central memory CD4+ T lymphocyte phenotype (CD45RA-CD28+), the strength of the association with age was reduced, while CD4+ T cell response category remained strongly significant after adjusting for these additional factors (95%CI -13.5 to 1.3, $P=0.11$ and 95%CI -22.5 to -7.9, $P<0.001$, respectively).

Discussion

Variability in CD8+ and CD4+ T Lymphocyte Marker Percentages in HIV-Infected Individuals

Our data demonstrate a high degree of variability of CD8+ and CD4+ T lymphocyte subsets in treatment-naïve HIV-infected individuals across the age ranges and CD4+ T lymphocyte response categories within our population. Despite this variability, we found that older age was associated with a higher frequency of senescent CD8+ T lymphocyte phenotypes (CD56+57+, CD56+, and CD28-), as well as a lower frequency of naïve CD8+ T lymphocytes (CD45RA+CD28+). This is consistent with studies of immune phenotypes found in very elderly individuals. Patients in our older subgroup with a mean age of 57.8 (range, 51–75 years) were immunologically similar to HIV-uninfected octogenarians and nonagenarians described in other studies [11, 16].

In univariate and multivariate analysis of CD4+ T lymphocyte phenotypes, younger individuals and CD4+ T cell responders had a higher proportion of naïve CD4+ T cells (CD45RA+CD28+). This is consistent with prior studies showing that the presence of naïve CD4+ T cells is associated with better immune reconstitution after ART initiation [22–24]. Additionally, consistent with at least one prior study [25], younger individuals had a lower proportion of CD28- senescent CD8+ T cells. Unlike our data, this study found a stronger association between age and loss of naïve CD8+ T cells compared with naïve CD4+ T cells, although both were reduced with aging. Taken together, these data provide further evidence to suggest that earlier treatment, particularly in older individuals, is likely to be beneficial in optimizing immune function and maximizing CD4+ T cell response to ART.

Older individuals and CD4+ T cell nonresponders were also found to have a higher baseline percentage of CD4+ central memory cells (CD45RA-CD28+), although the strength of the association with age was reduced in multivariate analysis. This is consistent with previous data showing that progression of HIV infection is associated with a depletion of naïve CD4+ T cells and a corresponding increase in intermediate and late-differentiated CD4+ T lymphocytes [21, 26]. In our sample, HIV patients with a larger pool of central memory CD4+ T cells had less robust response to ART, suggesting that central memory cells expanded to make up for the deficit of naïve cells.

Specific clinical trial data has suggested that a PI plus two NRTIs may be associated with a greater increase in CD4+ T lymphocyte counts compared with efavirenz plus two NRTIs at 96 weeks [27]. The prevalence of PI-containing regimens was not significantly different among the four groups we evaluated, and the presence of a PI in the ART regimen was not associated with an increased likelihood of being in the CD4+ T cell responder category (>200 cells/mm³), regardless of age.

Unexpectedly, significantly higher percentages of senescent CD56+CD57+ CD8+ and CD56+ CD8+ T cells were found in individuals with good CD4+ T lymphocyte responses to ART compared with those with poor responses, even after controlling for age, race, sex, regimen, and hepatitis status. This phenotype has been established as a senescent subset in

the Swedish NONA studies of elderly individuals [13, 17, 28, 29]. In our sample, the overall prevalence of these CD8+ T lymphocyte subsets was low, with a mean percentage of 4.2% for CD56+57+ and 6.2% for CD56+ across the sample. The unexpected higher percentage in individuals with these senescent markers with good CD4+ T cell responses was therefore seen in the setting of overall low prevalence of this subtype, making the significance difficult to interpret. Additionally, our study only examined PBMCs from the baseline pre-ART time-point. It is possible that, after ART initiation, the proportions of CD56+ and CD56+57+ T lymphocytes shifted and that subsequent on-ART PBMCs analysis could have revealed an association with higher proportions of these senescent CD8+ T cells and CD4+ nonresponders.

Possible Components of an Immune Risk Phenotype in HIV-Infected Individuals: Beyond T Lymphocyte Markers

It is likely that the CD4+ T lymphocyte response to potent ART is determined by multiple factors within a given individual, including nadir CD4+ T cell count, the presence of co-infections such as CMV, and the influence of chronic inflammation as measured by elevated inflammatory cytokines and biomarkers. This multi-faceted interaction has been called the inflammation-aging hypothesis and invokes a cycle of chronic T lymphocyte stimulation as the cause of “unsuccessful aging”, defined by the development of cardiovascular disease, cancers, osteopenia and osteoporosis, left ventricular dysfunction, liver failure, kidney failure, cognitive decline, and frailty [9]. Given the similarities between elderly and HIV-infected individuals, specifically chronic infections and immune activation, a similar cycle of inflammation-aging may apply. It is clear that even in the setting of viral suppression to <50 copies/ml in the blood compartment, immune activation persists [30], and at least one study has demonstrated that aged T cells continue to accumulate in patients on ART with long-term viral suppression [31].

Factors other than T lymphocyte surface markers that may better explain immune aging in HIV infection include other T cell characteristics of replicative senescence, such as telomere shortening, anergy, and resistance to apoptosis. Studies have shown that the loss of CD28 in chronic HIV infection is associated with a decline in telomerase activity and telomere length [32, 33]. Since telomere length shortens with each cell division, this process results in decreased proliferation and may lead to poor response to antigens. Supporting the importance of a role for telomere length in HIV progression are studies demonstrating high proliferative capacity among HIV-infected long-term nonprogressors [34, 35].

Finally, thymic function is reduced in older patients, and studies of HIV-infected individuals have shown that CD4+ T-cell-receptor-rearrangement excision circles, a marker of thymic output, are reduced in both older HIV-infected patients and those with late-stage disease [36]. Thymic function may be an important component of an IRP for older HIV-infected individuals, though it remains an elusive target for therapeutic intervention.

Study Limitations and Weaknesses

We analyzed pre-ART PBMCs to detect an IRP that might predict 96-week CD4+ T lymphocyte response to ART but did not look at CD8+ and CD4+ T lymphocyte phenotypes from other time points after the initiation of ART. It is known that in the setting of ART, lymphocyte phenotypes shift in response to viral suppression and decreased immune activation. T lymphocyte phenotypes at 4, 8, or 12 weeks after ART initiation may be more predictive of CD4+ T cell response than those phenotypes seen before treatment initiation.

We did not have the ability to collect information on CMV antibody status or the presence of CMV viremia. Prior research supports the important role of CMV in the IRP for elderly

patients, and while we presume high rates of CMV seropositivity in HIV-infected populations, data on serostatus and active (even low-grade) CMV viral replication would have further informed our study results.

Given previous data about the inflammation–aging hypothesis, measurement of pro-inflammatory cytokines and biomarkers measured in conjunction with T lymphocyte cell surface markers may provide a more comprehensive view of immune senescence. Additional measures of telomerase, anergy, and apoptosis may also prove to be critical in understanding the interaction of HIV and immune senescence and in the identification of an IRP for HIV-infected individuals. Finally, our study measured immune phenotypes from the blood compartment only. Viral load from blood plasma represents disease status from only one compartment, while ongoing HIV burden in the gut, lymphoid tissue, central nervous system, and genital tract is emerging as important in the pathogenesis of HIV and virus-specific immune responses [37].

The Role of Low PBMC Viability on Study Results

We encountered unexpected viability issues with the cryopreserved PBMCs utilized in our study. Forty-seven samples (30%) could not be used because cells were not viable, and an additional eleven samples were excluded because cell viability was less than 10%. The mean viability among the samples included was low at 44.3% (standard deviation 19.6%) as determined by DNA dye exclusion measured by flow cytometry, with no significant differences in the mean viability between the four groups (viability range 10.1–79.6%). The samples were selected from ACTG studies dating from 1998. It is possible that storage protocols were not optimal, particularly at the time of early ACTG studies when cell processing and freezing protocols were being refined. We chose to exclude samples that had less than 10% viability, and we could not detect statistically significant differences in baseline characteristics between those included versus those excluded. However, a higher proportion of low-viability and non-viable samples were from older individuals, such that samples included in the final analysis may not have been representative of T cell phenotypes in this population. It is possible that senescent cells would be more likely to be non-viable after prolonged cryopreservation.

Conclusion

HIV-infected younger individuals demonstrate a lower frequency of pre-ART senescent CD8+ T lymphocyte phenotypes (CD56+CD57+, CD56+, and CD28–) and a higher frequency of naïve CD8+ T cells (CD45RA+ CD28+) compared with older individuals. Our study also confirms other studies showing that older HIV-infected individuals and those with poor CD4+ T cell response to ART have lower baseline pre-ART frequencies of naïve CD4+ T lymphocytes, as well as a shift away from naïve and toward central memory CD4+ T lymphocytes. Our study was unique in that it attempted to determine if the IRP described previously in elderly populations could be replicated in HIV-infected patients and explain poor CD4+ T cell responses to ART. It is likely that an IRP in HIV-infected individuals will be defined not only by CD4+ and CD8+ T lymphocyte phenotypes but also by the presence of inflammatory cytokines and other biomarkers, markers of T cell replication capacity such as telomerase and telomere length, as well as the presence of co-infections. Ultimately, understanding factors associated with poor CD4+ T lymphocyte responses and defining an HIV-associated IRP may provide population targets for future therapies and lead to improved outcomes for HIV-infected patients.

Acknowledgments

We are grateful to the Claude D. Pepper Older Americans Independence Center (AG28748), the UCLA Division of Geriatric Medicine, UCLA Center for AIDS Research (AI28697), and the AIDS Clinical Trials Group (AI69424, AI68636, AI68634), and ACTG Longitudinal Linked Randomized Trials for their support of this project. We gratefully acknowledge Mary Ann Hausner and Roger Shih for their technical support in thawing and preparing the samples, and thank Janet Andersen for valuable input on the analyses.

Grant support Ron Mitsuyasu received support from the UCLA Older Americans Independence Center, NIH/NIA Grant P30-AG028748, and the content does not necessarily represent the official views of the National Institute on Aging or the National Institutes of Health. Additional support was provided by the following NIH grants: AG-028748, AI-069424, and AI-28697. The UCLA Flow Cytometry Core Facility is supported by grants NIH/NIAID AI028697 and NIH/NCI CA016042. B.D. Jamieson is supported by NIH/NIAID Grant AGO30327. The ACTG and ACTG Longitudinal Linked Randomized Trials are funded by NIH/NIAID grants: AI-68636, AI-68634, AI-38858, and AI-38855.

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Table 1

Characteristics of subjects included in analysis (>10% cell viability, n=100)

	Group 1 Older nonresponders (n=12)	Group 2 Younger nonresponders (n=31)	Group 3 Older responders (n=26)	Group 4 Younger responders (n=31)	P value [*]
Age in years mean (SD)	59.3 (7.0)	38.7 (4.7)	57.1 (4.9)	39.4 (5.9)	<0.001
Percent female (n)	25.0% (n=3)	16.1% (n=5)	34.6% (n=9)	19.4% (n=6)	0.39
Baseline CD4+ T cell count (cells/mm ³) mean (SD)	303 (222)	252 (127)	305 (201)	228 (162)	0.32
CD4+ T cell count (cells/mm ³) change from baseline to 96 weeks mean (SD)	49 (50)	38 (50)	381 (133)	333 (102)	<0.001
Baseline log ₁₀ RNA (copies/ml) mean (SD)	4.7 (0.5)	4.6 (0.7)	5.1 (0.8)	4.9 (0.7)	0.13
Hepatitis B surface antigen-positive	8.3% (n=1)	9.7% (n=3)	3.8% (n=1)	3.2% (n=1)	0.62
Hepatitis C virus antibody-positive	16.7% (n=2)	9.7% (n=3)	7.7% (n=2)	9.7% (n=3)	0.85
Non-white race	66.7% (n=8)	32.3% (n=10)	53.8% (n=14)	58.1% (n=18)	0.10
Protease inhibitor-containing ART ^a	41.7% (n=5)	22.6% (n=7)	34.6% (n=9)	32.3% (n=10)	0.59

^{*} P values determined by ANOVA for continuous variables and Fisher's exact test for categorical variables

^aThe remainder were on non-nucleoside reverse transcriptase-based regimens with the exception of 15% (n=15) individuals who were on only nucleoside reverse transcriptase inhibitor combination therapy

Table II

Comparison of patient characteristics of those included versus excluded in analysis at the 10% viability cut-off

Characteristic	Included (Viability >10%) <i>n</i> =100	Excluded (Viability ≤10%) <i>n</i> =58	<i>P</i> value *
Age (mean, years) (SD)	46.1 (10.6)	49.5 (10.7)	0.06
Baseline CD4+ T cell count (mean, cells/mm ³) (SD)	266 (172)	270 (222)	0.89
96 Week CD4+ T cell count (mean, cells/mm ³) (SD)	492 (252)	513 (261)	0.64
CD4+ T cell change from pre-ART baseline to 96 weeks (mean, cells/mm ³) (SD)	226 (182)	243 (312)	0.73
Baseline log ₁₀ RNA (copies/ml) (SD)	4.82 (0.7)	4.98 (0.9)	0.21

*
P values were determined from *t* tests

Table III
CD8+ T lymphocyte and CD4+ T lymphocyte marker expression: Age and Responder Effects^a

CD8+ T-Lymphocytes	Group 1 Older Nonresponder N=12	Group 2 Younger Nonresponder N=31	Group 3 Older Responder N=26	Group 4 Younger Responder N=31	Age Effect v. Older (referent) Estimate* ★95% CI, P-value	Responder Effect Nonresponder v. (referent) Estimate* ★95% CI, P-value
CD45RA+CD45RO+ CD8+ mean% **SD (IQR) (transitional CD8+ T-lymphocytes)	7.1 4.7 (5.6, 6.5)	8.1 4.4 (3.0, 12.2)	8.2 4.6 (3.7, 14.9)	7.8 7.8 (3.2, 13.1)	0.16 -1.8, 2.2 0.88	0.19 -1.8, 2.1 0.85
CD45RA+CD28- CD8+ mean% SD (IQR) (terminal effector)	32.0 7.8 (2.5, 10.7)	32.2 12.8 (2.9, 32.9)	38.4 6.2 (3.3, 18.2)	31.7 10.3 (2.6, 29.7)	-4.0 -10.0, 2.0 0.19	1.9 -4.0, 7.8 0.52
CD28- CD8+ mean% SD (IQR) (senescent marker)	72.9 10.4 (6.3, 80.7)	65.8 15.5 (4.2, 82.9)	72.3 12.0 (6.4, 83.7)	65.5 13.3 (4.9, 28.1)	-6.9 -12.5, -1.4 0.02	-0.41 -5.8, 5.0 0.88
CD56+CD57+ CD8+ mean% SD (IQR) (senescent marker)	3.0 2.6 (1.3, 3.2)	2.7 2.2 (0.5, 4.6)	6.7 6.7 (1.6, 15.2)	3.9 3.8 (0.8, 8.0)	-1.8 -3.6, -0.02 0.047	2.1 0.31, 3.8 0.02
CD57+CD28- CD8+ mean% SD (IQR) (senescent marker)	29.7 14.4 (20.1, 39.4)	32.6 16.1 (13.2, 54.6)	40.2 17.0 (18.7, 64.4)	33.9 13.1 (20.3, 55.0)	-2.8 -9.2, 3.6 0.39	4.5 -1.8, 10.7 0.16
CD56+ CD8+ mean% SD (IQR) (senescent marker)	5.8 4.9 (2.1, 8.3)	4.3 2.6 (1.4, 7.9)	8.6 7.5 (2.7, 16.6)	6.2 4.5 (1.4, 12.8)	-2.1 -4.2, -0.03 0.05	2.2 0.17, 4.3 0.03
CD57+ CD8+ mean% SD (IQR) (senescent marker)	33.1 14.5 (22.2, 44.1)	36.3 16.7 (16.1, 61.2)	43.1 17.1 (20.7, 65.0)	36.5 13.0 (23.0, 56.0)	-2.9 -9.4, 3.6 0.37	3.6 -2.8, 9.9 0.27
CD45RA+CD28+ CD8+ mean% SD (IQR) (naïve)	8.5 7.8 (2.5, 10.7)	13.9 12.8 (2.9, 32.9)	9.0 6.2 (3.3, 18.2)	16.6 10.3 (2.6, 29.7)	6.8 2.6, 10.9 0.002	1.9 -2.2, 6.0 0.35
CD45RA-CD28+ CD8+ mean% SD (IQR) (central memory)	18.6 6.9 (14.4, 20.8)	20.3 9.2 (9.8, 31.6)	18.8 11.1 (8.6, 24.3)	17.9 7.8 (10.1, 27.7)	0.13 -3.6, 3.9 0.95	-1.5 -5.2, 2.2 0.42
CD45RA-CD28- CD8+ mean% SD (IQR) (effector memory)	40.9 17.1 (26.7, 53.0)	33.6 17.4 (9.6, 55.6)	33.9 14.2 (16.4, 51.0)	33.8 16.8 (14.0, 60.5)	-2.9 -9.7, 4.0 0.41	-2.3 -9.0, 4.4 0.49

CD4+CD8+ T-Lymphocytes	Group 1 Older Nonresponder N=12	Group 2 Younger Nonresponder N=31	Group 3 Older Responder N=26	Group 4 Younger Responder N=31	Age Effect Younger v. Older (referent) Estimate ★95% CI, P-value	Responder Effect Responder v. Nonresponder (referent) Estimate ★95% CI, P-value
CD3+CD4+ CD8+ mean% SD (IQR) (dual positive/senescent marker)	0.4 0.2 (0.2, 0.4)	0.4 0.2 (0.1, 0.6)	0.3 0.4 (0.1, 0.7)	0.2 0.2 (0.1, 0.4)	-0.07 -0.18, 0.03 0.16	-0.05 -0.15, 0.05 0.36

CD4+ T-Lymphocytes	Older Nonresponder N=12	Younger Nonresponder N=31	Older Responder N=26	Younger Responder N=31	Age Effect Younger v. Older (referent) Estimate ★95% CI, P-value	Responder Effect Responder v. Nonresponder (referent) Estimate ★95% CI, P-value
CD45RA+CD28+ CD4+ mean% SD (IQR) (naïve)	11.4 12.6 (2.8, 14.4)	21.5 17.4 (4.3, 49.4)	20.8 16.0 (2.1, 40.3)	29.5 19.9 (2.5, 55.3)	9.2 2.0, 16.4 0.01	8.5 1.5, 15.6 0.02
CD45RA-CD28+ CD4+ mean% SD (IQR) (central memory)	78.7 13.3 (72.2, 87.7)	65.1 18.3 (43.3, 85.6)	58.7 23.3 (35.0, 81.6)	54.3 15.8 (31.5, 69.9)	-7.9 -15.6, -0.17 0.045	-14.0 -21.5, -6.4 < 0.001
CD45RA-CD28- CD4+ mean% SD (IQR) (effector memory)	8.6 10.0 (3.7, 7.8)	10.3 11.9 (0.4, 25.9)	14.9 20.7 (0.9, 29.9)	12.4 17.1 (0.9, 31.6)	-0.83 -7.5, 5.8 0.81	3.5 -3.0, 10.1 0.28
CD45RA+CD28- CD4+ mean% SD (IQR) (terminal effector)	1.5 3.5 (0.1, 0.7)	3.1 4.7 (0, 10.0)	5.6 8.4 (0, 12.2)	3.7 6.3 (0.1, 14.3)	-0.53 -3.1, 2.1 0.69	1.8 -0.72, 4.4 0.16
CD28- CD4+ mean% SD (IQR) (senescent marker)	10.0 10.2 (4.4, 13.4)	13.4 15.0 (0.6, 38.6)	20.4 25.0 (1.2, 40.7)	16.1 20.4 (1.4, 55.9)	1.4 -6.7, 9.4 0.74	5.4 -2.5, 13.3 0.18

^ Data reported for all samples with viability > 10% (n=100)

* Based on linear regression models for each marker that include effects for both age and responder categories. Separate modeling showed no evidence for an interaction between the age and responder effects for any CD4+ or CD8+ T-lymphocyte subpopulation (all $P \geq 0.15$)

** SD, standard deviation. IQR: interquartile range ★95% confidence intervals are adjusted and represent the difference from the referent