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Changes in Markers of T-Cell Senescence and Exhaustion With Atazanavir-, Raltegravir-, and Darunavir-Based Initial Antiviral Therapy: ACTG 5260s

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It is unclear whether differential roles of $CD4^+$ versus $CD8^+$ T-cell senescence/exhaustion and effects of antiretroviral therapy (ART) on these processes may contribute to morbidity in treated human immunodeficiency virus type 1 (HIV) infection. In a prospective 96-week trial, 328 HIV–infected ART-naive participants were randomly assigned to receive tenofovir-emtricitabine plus either atazanavir/ritonavir, darunavir/ritonavir, or raltegravir. Markers of $CD4^+$ T-cell senescence (ie, the percentage of $CD28^-CD57^+$ cells among $CD4^+$ T cells) and $CD4^+/CD8^+$ T-cell exhaustion (ie, the percentage of $PD-1^+$ cells among $CD4^+/CD8^+$ T cells) decreased after ART. There were no changes in markers of $CD8^+$ T-cell senescence after ART and no differential changes in all markers in ART groups. Senescent $CD4^+$ and $CD8^+$ T cells may have differential roles in HIV pathogenesis.

Keywords.   antiretroviral therapy; human immunodeficiency virus; inflammation; immune activation; biomarkers.

Human immunodeficiency virus type 1 (HIV) infection is characterized by a state of inflammation and immune activation that persists despite suppressive antiretroviral therapy (ART) and may contribute to the development of end-organ diseases such as cardiovascular disease (CVD) [1]. Chronic HIV infection is characterized by T-cell senescence, a terminal state with dysregulated immune function and production of proinflammatory cytokines [2]. Senescent T cells may increase the risk of morbidity, such as CVD [3], but data from individuals with HIV infection are limited and controversial [4, 5].

The mechanisms driving T-cell exhaustion, immunosenescence, activation, and chronic comorbidities during chronic HIV infection are not completely understood. In addition, although we and others have found that effective ART only partially decreases immune activation and inflammation [1], limited data exist on the effect of ART initiation in the setting of a randomized trial. Furthermore, it is unclear whether changes in these markers would differ in the setting of an integrase inhibitor–based regimen, compared with boosted protease inhibitor (PI) therapy.

AIDS Clinical Trials Group (ACTG) A5260s was a prospective cardiovascular substudy of ACTG A5257, in which participants were randomly assigned equally to receive one of the following 3 regimens: tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) plus either an integrase-based regimen containing raltegravir (RAL), a PI-based regimen containing atazanavir (ATV)/ritonavir (r), or TDF/FTC plus darunavir (DRV)/r [1]. In this study, we found that ATV/r was associated with slower progression of carotid intima media thickness, compared with DRV/r or RAL, and that levels of markers of inflammation and T-cell activation inconsistently and partially decreased after these regimens [1], without a consistent advantage for any of the regimens.

The objective of this analysis was to characterize the changes in biomarkers of T-cell senescence longitudinally among treatment-naive individuals initiating these randomly assigned ART regimens. In this article, we describe for the first time the effects of different successful ART regimens on T-cell senescence and exhaustion in the setting of a large randomized ART initiation trial. In addition, associations between key biomarkers of T-cell exhaustion, immune senescence and activation, and inflammation were explored in ART-naive HIV-infected participants at study entry and through week 96 of a successful ART regimen.

METHODS

Study Design and Participants

A5260s included 328 HIV-infected, ART-naive adults with no CVD or diabetes mellitus or use of lipid-lowering medications. The design of this substudy has been previously reported [1], as well as key results related to cardiovascular and bone end points [6, 7]. The parent study and substudy (ClinicalTrials.gov identifiers NCT00811954 and NCT00851799) were approved by the local institutional review boards. For this preplanned analysis, to avoid the confounding effect of viremia on markers of immunosenescence and exhaustion, the A5260s population was restricted to a subset of participants with virological suppression, no ART interruptions lasting >7 days, and an HIV RNA level of < 50 copies/mL by study week 24 and thereafter.

Biomarker and Laboratory Assessment

Blood samples were collected at study entry, prior to ART initiation, and after 24 and 96 weeks of treatment. Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved and then underwent immunophenotyping by multicolor flow cytometry as described previously [1, 6]. The fluorochrome-conjugated antibodies used were anti-CD3 PE-Cy7 (clone SK7), anti-CD4 V450 (clone RPA-T4), anti-CD8 APC (clone RPA-T8), anti-CD8 APC-Cy7 (clone SK1), anti-CD28 PE-Cy 5 (clone CD28.2), anti-CD57 PE (clone NK-1), and anti- CD279 (PD-1) APC (clone MIH4), all from BD Biosciences. A representative gating strategy is shown in Supplementary Figure 1.

Statistical Analyses

Changes in biomarker values over time were estimated by determining the mean difference between levels during treatment and the level at baseline on the $\log_{10}$ scale and then back transforming the values to represent mean fold changes from baseline. For all pair-wise treatment group comparisons, shifts in the distribution of change from baseline were evaluated using Wilcoxon rank sum tests and are described as relative fold changes. While nominal P values are presented, adjusted P values, determined using Benjamini-Hochberg methods to control the false discovery rate, are also provided to acknowledge comparisons across the multiple biomarkers at each study week. Exploratory bivariate associations, quantified with Spearman rank correlations, were examined at concurrent study weeks between levels and changes in biomarkers. All analyses were performed with SAS, version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

Baseline Characteristics

Baseline demographic characteristics of the 328 participants from the A5260s study were previously described [1]. The 234 participants (71%) included in the virologically suppressed population for this analysis had similar baseline demographic characteristics across treatment groups, as previously described [1]. Briefly, 90% were men, 48% were white, the median age was

36 years, the median CD4$^+$ T-cell count was 338 cells/mm^3, and the median HIV RNA level was 4.6 $\log_{10}$ copies/mL. Baseline levels of all biomarker parameters are presented in Table 1. No notable differences in distributions were apparent between treatment groups.

Changes in Markers of Immunosenescence and Exhaustion

Sustained decreases (of about 31% or more) from baseline in the percentage of CD28$^-$CD57$^+$ cells among CD4$^+$ T cells were evident among all treatment groups (Figure 1 and Table 1). In contrast, there was no apparent change from baseline to 96 weeks in the percentage of CD28$^-$CD57$^+$ cells among CD8$^+$ T cells. For exhaustion markers, declines from baseline were evident for the percentage of PD-1$^+$ cells among both CD4$^+$ and CD8$^+$ T cells across all treatment groups, by week 24, with further apparent declines for CD4$^+$ T-cell exhaustion by week 96; week 96 levels were on average at least 56% lower than baseline levels. Differences by treatment group were not apparent for any immunosenescence or exhaustion marker ($P > .1$; Supplementary Table 1). Overall, sustained declines over time were evident in all treatment groups for CD4$^+$ T-cell markers of senescence and exhaustion.

Correlations Between Biomarkers of T-Cell Senescence, Exhaustion, Inflammation, and Immune Activation

At baseline, plasma viral load was correlated with markers of exhaustion (PD-1$^+$; r = 0.24–0.31; $P < .001$) but not senescence ($|r| < 0.15$, $P > .05$). With regard to associations of baseline markers of immunosenescence with markers of T-cell and monocyte activation or of inflammation at baseline (Supplementary Table 2), the strongest associations were apparent between the percentage of CD38$^+$DR$^+$ cells among CD4$^+$ T cells and the percentages of CD28$^-$CD57$^+$ cells (r = 0.42) and PD-1$^+$ cells (r = 0.40) among CD4$^+$ T cells (Supplementary Figure 2). There was also a moderate association at baseline between monocyte activation (sCD163) and CD4$^+$ T-cell exhaustion (ie, the percentage of PD-1$^+$ cells among CD4$^+$ T cells; r = 0.35). These moderate associations were also apparent when examining the concurrent changes over 96 weeks between these set of markers, with correlations ranging from 0.33 to 0.55

Table 1. Baseline Values and Fold Changes From Baseline Over Time for 212 Study Participants, by Treatment Group

Biomarkers	Baseline, Mean (95% CI[a]) (n = 58)	ATV/r (n = 64), Mean (95% CI[a])		RAL (n = 72), Mean (95% CI[a])		DRV/r (n = 76), Mean (95% CI[a])	
		Week 24 (n = 58)	Week 96 (n = 61)	Week 24 (n = 65)	Week 96 (n = 70)	Week 24 (n = 73)	Week 96 (n = 72)
Immunosenescence, CD28$^-$CD57$^+$ cells, %							
Among CD8$^+$ T cells	24.35 (17.8,30.75)	1.04 (0.91,1.19)	0.96 (0.85,1.09)	1.15 (1.07,1.23)	1.07 (0.98,1.17)	1.13 (1.07,1.20)	0.99 (0.89,1.10)
Among CD4$^+$ T cells	5.01 (2.24,9.97)	0.73 (0.60,0.90)	0.66 (0.52,0.85)	0.85 (0.72,1.00)	0.76 (0.62,0.93)	0.85 (0.74,0.97)	0.65 (0.54,0.79)
Cell exhaustion, PD-1$^+$ cells, %							
Among CD8$^+$ T cells	2.33 (1.48,3.87)	0.43 (0.34,0.55)	0.42 (0.33,0.54)	0.42 (0.36,0.49)	0.33 (0.27,0.40)	0.35 (0.30,0.42)	0.32 (0.24,0.43)
Among CD4$^+$ T cells	4.37 (2.57,7.62)	0.58 (0.48,0.70)	0.38 (0.30,0.47)	0.60 (0.50,0.70)	0.44 (0.37,0.52)	0.50 (0.44,0.58)	0.39 (0.32,0.48)

Data are for participants with information on biomarkers available at the specified time points for calculations of changes.

Abbreviations: ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; RAL, raltegravir.

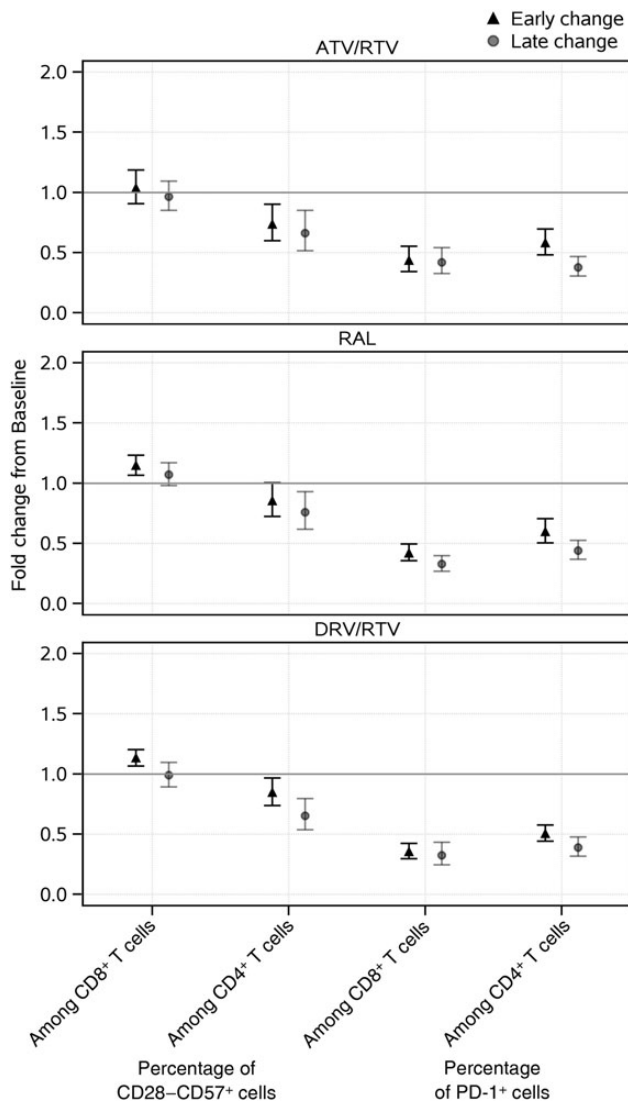[a] 95% confidence intervals (CIs) excluding 1.0 are considered statistically significant.

Figure 1. Early and late changes in immunosenescence markers, by treatment arm. Point estimates and error bars reflect mean and 95% confidence intervals, respectively. Early change represents change from baseline to week 24; late change represents change from baseline to week 96. Abbreviations: ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; RAL, raltegravir; RTV, ritonavir.

(Supplementary Table 3); in contrast, a moderate association between the levels of these markers at week 96 was only apparent for CD4+ T-cell activation and senescence (r = 0.44). All remaining associations between biomarkers were not apparent or were weak (|r|<0.3; Supplementary Tables 2, 3).

DISCUSSION

In this prospective study of ART-naive participants who achieved virological suppression after initiation of TDF/FTC along with RAL, ATV/r, or DRV/r, RAL did not have a more favorable effect on decreasing immunosenescence or exhaustion as compared to the PIs. To our knowledge, this is the largest prospective study describing changes in markers of immune senescence and exhaustion after initiation of successful ART. We also describe associations with markers of immune activation and inflammation that have been associated with serious clinical events in HIV–infected persons, including CVD and mortality [8]. Of note, although sustained and similar declines over time from baseline were evident in all treatment groups for all CD4+ T-cell markers of senescence and exhaustion, consistent reductions in markers of CD8+ T-cell senescence were not apparent. Overall, these results add to the current literature outlining the incomplete reversal of inflammation, senescence, and immune activation in the setting of effective treatment [9].

Understanding how different initial ART regimens reduce chronic immune dysfunction in successfully treated HIV infection is an ongoing research priority. Other studies have suggested that integrase inhibitors may reduce inflammation and viral load more effectively than other antiretroviral agents [10, 11]. Intensification with the integrase inhibitor RAL has shown variable effects on immune activation [10]. Despite this limited evidence, the differential effects of integrase inhibitors as compared to other ART agents on T-cell senescence remain unknown. We did not find any benefit of RAL over PIs on reducing immunosenescence or exhaustion during the first 96 weeks of successful treatment. This is consistent with a study by Kaplan et al, in which no differential effect of PIs were seen as compared to nonnucleoside reverse-transcriptase inhibitors on T-cell senescence [4]. A strength of our study is that, in contrast to prior RAL switch or intensification-related studies, our data are not confounded by prior ART.

The age-associated remodeling of the immune system through accumulation of senescent T cells may contribute to numerous aging-related pathologies [2]. Senescent T cells have been associated with CVD [3], but the differential role of CD4+ T-cell senescence as compared to CD8+ T-cell senescence in the development of chronic comorbidities such as CVD and bone disease remain unclear. CD8+ T-cell senescence has been associated with progression of numerous diseases [2], including CVD, in a study of HIV-infected patients [4], but this was not confirmed by others [5]. Senescent CD4+ T cells are rare, long-lived senescent T cells with proatherogenic properties [12] that may result from persistent antigenic stimulation [12] and play a critical role in the development of age-related pathologies [2]. HIV-infected patients with a low CD4+ T-cell count during ART have a greater number of senescent CD4+ T cells than those with increased CD4+ T-cell levels [13]. The lack of change in markers of CD8+ T-cell senescence in our study may be due to our cohort having a relatively high overall pre-ART CD4+ T-cell count and, perhaps, a lower overall pre-ART level of senescent CD8+ T cells, compared with cohorts in other studies [4, 5, 9]. Together, these prior studies highlight possible differential roles of senescent CD4+ T cells and senescent CD8+ T cells [14] in the pathogenesis of HIV and its comorbidities and are consistent

with our results that effective ART differentially reduced CD4$^+$ (and not CD8$^+$) T-cell senescence.

In view of the limited data regarding the interplay between T-cell exhaustion, senescence, inflammation, and immune activation in chronic HIV infection, we found modest bivariate associations only between biomarkers of CD4$^+$ and CD8$^+$ T-cell activation and exhaustion at baseline and changes over 96 weeks. At week 96, these associations were apparent for CD4$^+$ but not CD8$^+$ T-cell activation and exhaustion biomarkers. The only other moderate association detected was between baseline biomarkers of CD8$^+$ T-cell exhaustion and sCD163, a biomarker of monocyte activation that has been associated with CVD [15]. This finding may be explained by the fact that normal CD4$^+$ T cells orchestrate multiple signals from T cells, B cells, and antigen-presenting cells and that senescent CD4$^+$ T cells contribute to the pathogenesis of states of immune activation, such as autoimmunity [2]. Further studies are needed to elucidate the cross-talk between HIV infection, T-cell activation, T-cell senescence, and T-cell exhaustion and chronic comorbidities, such as CVD and bone disease.

Our study had several limitations that have previously been described. Briefly these include limited power to detect effect sizes with adjustment for multiple biomarker comparisons, selection bias of A5260s participants when restricting analysis to the cohort of virologically suppressed individuals who continued to receive ART, and inclusion of mostly men, which may limit generalizability of our findings. Finally, the relatively younger age of our study population with no CVD may have limited our ability to study the cross-talk between T-cell senescence, exhaustion, and immune activation.

In conclusion, in this prospective study we did not find differential changes in T-cell senescence and exhaustion after 96 weeks among treatment-naive individuals initiating and continuing to receive successful ART regimens of TDF/FTC with RAL, ATV/r, or DRV/r. Despite successful ART therapy, markers of CD8$^+$ T-cell senescence did not decline; increased CD4$^+$ T-cell activation was associated with increased CD4$^+$ T-cell exhaustion and senescence. Overall, these data suggest differential roles of senescent CD4$^+$ and CD8$^+$ T cells in HIV immunopathogenesis in the setting of effective ART. Furthermore, these results also highlight the need to further understand the role of T-cell senescence and exhaustion in chronic HIV infection that may lead to adjunct ART regimens with more-potent antiinflammatory effects as a strategy to prevent chronic comorbidities associated with HIV infection.

STUDY GROUP MEMBERS

ACTG 5260s team members are as follows: H. Hodis, C. Godfrey, B. Jarocki, A. Benns, and K. Braun.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

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