HIV CONTROLLERS MAINTAIN VIRAL SUPPRESSION DESPITE WANING T-CELL RESPONSES ON ART

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Summary

In a large cohort of HIV controllers (ACTG A5308), HIV-specific T-cell responses in non-viremic controllers were higher than in viremic controllers. Despite decreasing T-cell responses on ART, controller status was preserved after therapy was stopped.

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Conflicts of Interest:

Dr. Li has received research support and consulted for Gilead Sciences, Merck Abbvie and Jan Biotech.

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ABSTRACT

AIDS Clinical Trials Group study A5308 found reduced T-cell activation and exhaustion in HIV controllers initiating antiretroviral therapy (ART). We further assessed HIV-specific T-cell responses and post-ART viral loads. Pre-ART, the 31% of participants with persistently undetectable viremia had more robust HIV-specific T-cell responses. On ART, significant decreases were observed in a broad range of T-cell responses. Eight controllers in A5308 and the SCOPE cohort showed no viremia above the level of quantification in the first 12 weeks after ART discontinuation. ART significantly reduced HIV-specific T-cell responses in HIV controllers but did not adversely affect controller status after ART discontinuation.

BACKGROUND

Spontaneous HIV controllers maintain low levels of HIV RNA in the absence of antiretroviral therapy (ART). Clinical trials like START conclusively demonstrated benefits of ART [1], but HIV controllers were not adequately represented in therapy trials and ART in controllers remains controversial [2, 3]. In the A5308 trial, the AIDS Clinical Trials Group (ACTG) demonstrated that ART in treatment-naïve HIV controllers suppressed already-low levels of viremia and led to significant improvements in T-cell activation and exhaustion suggesting possible benefits of ART in this population [4]. Robust T-cell responses are a hallmark of HIV controllers and appear critical for viral control [5-8]. However, the impact of ART on T-cell responses in controllers remains under-explored, leading to the concern that waning T-cell responses on ART might have adverse effects on controller status in those who subsequently stop ART. We assessed longitudinal changes of HIV-specific CD4+ and CD8+ T-cell responses for HIV controllers who initiated ART in A5308. Moreover, we describe virological outcomes after ART was discontinued in HIV controllers from both A5308 and the UCSF SCOPE cohort.

METHODS

A5308 Study Design and Population

ACTG A5308 was a prospective, open-label, multicenter study of rilpivirine/emtricitabine/tenofovir disoproxil fumarate (RPV/FTC/TDF) in ART-naïve HIV controllers [4]. Study design and participants were as described in Li *et al.* (Supplemental Fig. 1, Supplemental Table 1) [4]. HIV controllers qualifying as participants were ART-naïve (received ≤7 days of ART) with HIV-1 viral loads <500 copies/mL for ≥12 months. Viral loads ≥500 but <1,000 copies/mL were permitted if they represented fewer than half of the total measurements over the prior 12 months. In brief, participants remained off ART during a 12-week lead-in period and then received RPV/FTC/TDF for 48 weeks (Step 1), then were monitored for an additional 48 weeks in Step 2, during which they could choose whether to continue ART.

SCOPE Study Participants

The University of California, San Francisco (UCSF) SCOPE cohort is a prospective study of HIV-1 infected volunteers. We screened the SCOPE database for HIV controllers who met similar criteria as the A5308 participants: 1) ≥2 documented viral loads <500 copies/mL during the last 12 months before first initiation of ART, 2) ≥ one timepoint >12 months prior to ART with VL <1,000 copies/mL, 3) had received ART for ≥16 weeks, and, 4) were monitored for ≥ 16 weeks after ART discontinuation.

Viremia Assays

Plasma viremia was quantified by the Abbott RealTime HIV-1 viral load assay. Residual viremia was determined by the ultrasensitive integrase single-copy assay (iSCA) as described [4].

T-cell Phenotyping by Flow Cytometry

HIV-1-specific T-cell responses were measured by intracellular cytokine staining assays. Previously cryopreserved PBMCs were rested in R10 medium for 5 hours at 37°C in 5% CO_2,then incubated with an HIV-1 consensus clade B Gag peptide pool or SEB at 0.4 µg/mL (Sigma-Aldrich) as a control in the presence of anti-CD28 and anti-CD49d at 1 µg/mL (BD Bioscience) and antibodies against CD107a and -b (clones H4A3 and H4B4, respectively, BD Bioscience). After 1 hour, brefeldin A (5 µg/mL, BioLegend) and monensin (1 µg/mL, BD Bioscience) were added, and cells incubated for an additional 12 hours. An unstimulated negative control lacking antigenic peptides was included. Cells were washed and stained with Live/Dead Blue Viability Dye (Invitrogen), pre-incubated with 2 µL of FcR blocking reagent and surface-stained with antibodies against CD4 (RPA-T4; BD Bioscience), CD8a (SK1, BioLegend), and CD3 (OKT3, BioLegend). After a 20 min incubation, cells were washed, fixed, and permeabilized using the Cytofix/Cytoperm solution kit (BD Bioscience) for 30 min at 4°C. Intracellular cytokine staining was performed with antibodies against IFN-γ (4S.B3; BioLegend), interleukin-2 (IL-2; MQ1-17H12, BioLegend), and tumor necrosis factor alpha (TNF-α; MAb11, BioLegend), for 30 min at 4°C. Subsequently, cells were fixed in 2% paraformaldehyde in PBS and acquired on a BD 5LSR Fortessa cytometer (BD Bioscience). Unstimulated controls were used for background subtraction. Data were analyzed using FlowJo v.10.3 software (Tree Star LLC).

Statistical Analyses

Analyses of changes from baseline after 24-48 weeks of ART were based on the estimated treatment effect from a repeated measures analysis using general estimating equations (GEE).

Exact Wilcoxon rank-sum testing was performed for cross-sectional analysis of continuous outcomes by groups. No adjustments were made for multiple comparisons.

RESULTS

In A5308, 38 participants were enrolled from 19 ACTG-affiliated clinical research sites; 36 were started on ART, and 35 received at least 24 weeks of ART. Forty-three percent were female. When starting ART (week 0), 37% of individuals were suppressed by the Abbott Realtime HIV-1 RNA assay, the median CD4+ T-cell count was 682 cells/mm^3 [4].

Before ART, higher levels of HIV-specific CD4+ and CD8+ T-cell responses were found with viremia below the threshold of quantification both by the Abbott Realtime HIV-1 viral load assay and the iSCA (Supplemental Tables 2-5; corresponding SEB responses in Supplemental Tables 6-9). After 24-48 weeks of ART, significant decreases were observed in a broad range of HIV-specific CD4+ and CD8+ T-cell responses compared to pre-ART values (Fig. 1): these included CD4+ T-cells expressing IFNγ (-0.32% [95% confidence interval: -0.50%, -0.14%], p<0.001), IL-2 (-0.19% [-0.37%, -0.02%], p=0.03), and CD8$^+$ T-cells expressing IFNγ (-0.23% [-0.47%, 0%], p=0.05), TNFα (-0.32% [-0.58%, -0.07%], p=0.01). Furthermore, significant reductions were found in the percentages of polyfunctional HIV-specific CD4+ and CD8+ T-cells expressing multiple cytokines (CD4+ IFNγ+ TNFα+ CD107+: -0.08% [-0.14, -0.03], p=0.004; CD8+ IFNγ+ TNFα+ CD107+: -0.13% [-0.2, -0.05], p=0.001). Stratification showed declines in HIV-specific T cell responses for both viremic and non-viremic controllers (Supplemental Tables 10-15).

Four controllers in A5308 discontinued ART after 74, 86, 16, and 34 weeks, respectively, and were subsequently monitored off ART for at least 12 weeks. The participant who discontinued ART after 16 weeks was included in the present analysis, but did not reach the primary endpoint of 24 weeks on ART, hence was not included in the primary analysis of A5308 [4]. In addition, we identified 4 HIV controllers from the UCSF SCOPE study who received raltegravir/emtricitabine/tenofovir disoproxil fumarate (RAL/FTC/TDF) in a previous trial for a

median [Q1, Q3] of 33 [25, 65] weeks before stopping and for which we have extended follow-up[9]. Within 24 weeks after ART discontinuation, only one of the 8 controllers who had stopped ART in either the A5308 or SCOPE study had a transient viral load above the limit of quantification (Fig. 2) on the Abbott assay, but was suppressed with two subsequent viral loads. Viral loads measured by iSCA are available for three of the four A5308 participants who stopped ART, two of them show low-level viral loads prior to ART (Supplemental Fig. 3). Two SCOPE participants had transient viral loads more than 24 weeks after stopping ART but were virologically suppressed at the last available timepoints, which either represented the overall last available timepoint or time when ART was resumed (Fig. 2B, Supplemental Fig. 3). For the 4 SCOPE participants we now show maintenance of HIV control off ART for up to 6 years (122-304 weeks), considerably expanding previously reported times of post-ART viral suppression [9].

HIV-specific T-cell responses after stopping ART for the 3 A5308 participants who reached the primary study endpoint showed no clear trends after discontinuation of treatment (Supplemental Fig. 2).

DISCUSSION

ART has been insufficiently characterized in HIV controllers and remains controversial in this group [2, 3]. Nevertheless, HIV controllers are at risk for loss of viral control, declining CD4+ T-cell numbers, immune exhaustion, cardiovascular disease and other non-opportunistic conditions, possibly preventable by ART [4, 10].

Therefore, we assessed the effect of ART on HIV-specific T-cell responses in A5308, the largest prospective study to date of HIV controllers on ART. Prior to ART, controllers with undetectable viremia had more robust HIV-specific T-cell responses, underlining the importance of cell-mediated immunity for spontaneous HIV control [7, 8, 10]. In non-controllers, HIV-specific CD8+ T-cell responses decline rapidly within the first weeks on ART, then wane more slowly over the following 2 years [7, 8, 11]. Similarly, we detected decreasing CD4+ and CD8+ T-cell responses in HIV controllers on ART (Fig. 1). A trend in decreases in SEB T-cell responses may reflect a general decline in immune activation, and these trends appear different from the HIV-specific responses. In contrast, in some studies, ART in non-controllers led to increasing T-cell responses [12]. We hypothesize that the roles and kinetics of T-cell responses are fundamentally different in these individuals with advanced disease due to impairment of the immune responses, which may partially be restored on ART. Strong T-cell responses in non-viremic controllers that subsequently decrease on ART, as seen here, could reflect an efficient response to very low-level, undetectable viremia while viremic controllers may be individuals with slightly less robust responses, potentially due to some degree of immune exhaustion [4]. Persistent low-level viral replication in controllers may explain waning T-cell responses after antigen deprivation on ART. We hypothesize that despite decreasing T-cell responses on ART, T-cell memory remains functional allowing or persistent viral control after treatment interruption. Additional studies of post-ART time points are needed. On ART, we found significant decreases of both mono- and polyfunctional HIV-specific

T-cell responses. Nevertheless, participants from both A5308 and the SCOPE cohort maintained viral control after discontinuing ART.

A prior study including 16 HIV controllers initiating ART did not show significant changes in T-cell responses, although there was a trend towards a decrease in the percentage of Gag-specific IFNγ+ IL-2+ CD8+ T-cells [9]. In addition to the smaller sample number, the analysis of T-cell responses in that study differs from our work in that it was limited it to IFNγ+ IL-2+ T-cells only.

Given the overall significance of HIV-specific T-cell responses in maintaining HIV control and the waning responses we describe in HIV controllers who initiated ART, we further assessed whether ART use may adversely affect controller status. In non-controllers, discontinuation of treatment typically causes viral rebound within 4 weeks [13]. Chun *et al.* described 4 HIV controllers on ART for 24 weeks, then monitored off therapy for 3 months [14]: three individuals remained suppressed after stopping ART, and one participant, a viremic controller, showed a single viral load of 140 copies/mL 3 months after treatment interruption. No further viral load data are available, leaving unclear whether this reflects persistent loss of control or rather transient viremia which this individual had also experienced pre-ART. Our study aggregates data from A5308 and 4 participants from the SCOPE cohort who were on ART in a previously reported study of HIV controllers and for who we now provide extended follow-up [9]. Viremic episodes detected in a subset of participants were single, transient events, and HIV was subsequently suppressed in all 8 participants off ART. We believe these results provide reassurance for HIV controllers who are contemplating therapy as they seem to retain their controller status even after stopping ART.

Limitations of our study include the use of specific ART regimens. Monitoring on therapy was for up to 2 years , so we cannot exclude different outcomes after longer treatment duration. There is, however, evidence that HIV-specific T-cell responses in chronically-treated non-controllers, after an initial decrease, stabilize and are preserved [15]. Additional studies are needed to assess the long-term implications of ART in HIV controllers, especially for non-viremic controllers, for whom our numbers are still relatively limited. Three of the 8 participants who discontinued ART had transient viremia, potentially reflecting stochastic viral blips or reversion to a pre-ART phenotype with tendency for intermittent viremia In addition to reporting results on 4 HIV controllers that had stopped ART and were not identified previously, we provide extended follow-up on the 4 controllers off ART that were previously reported and part of the SCOPE cohort [9]. We monitored viral loads clinically but did not test antiviral activity of CD8+ T-cells, NK cells or antibody-dependent cellular cytotoxicity (ADCC) *in vitro*.

In summary, we report the largest study of HIV-specific T-cell responses in HIV controllers initiating ART and virologic outcomes after ART discontinuation. We show that ART significantly decreases HIV-specific T-cell responses. Nevertheless, the results provide reassurance for HIV controllers as they do not appear to lose their controller status with ART discontinuation.

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FIGURES

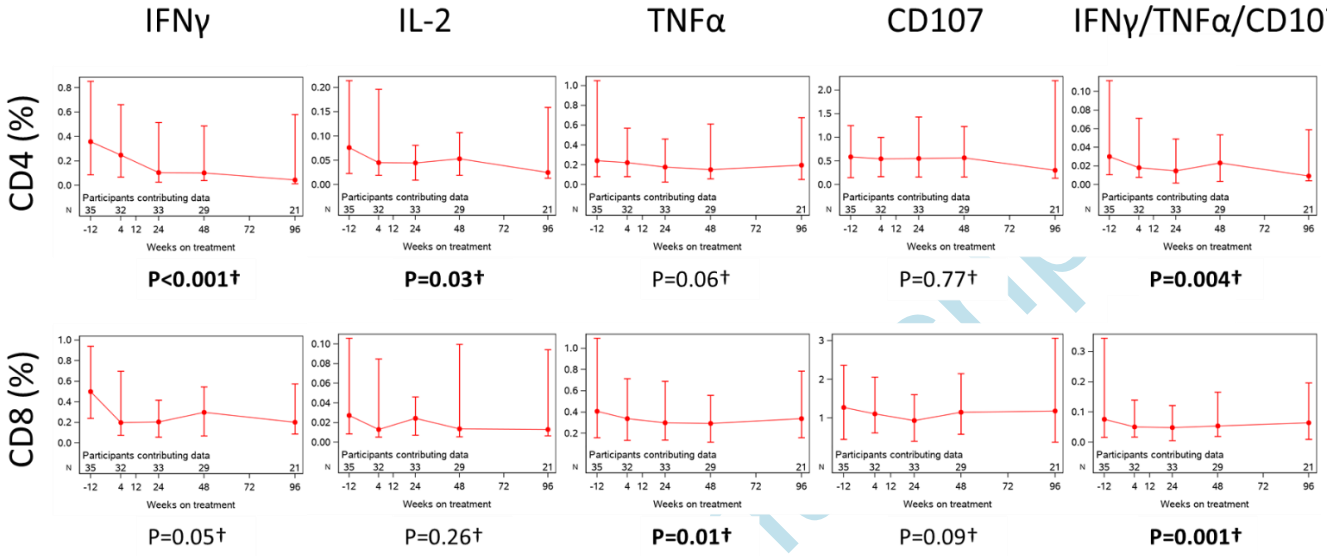
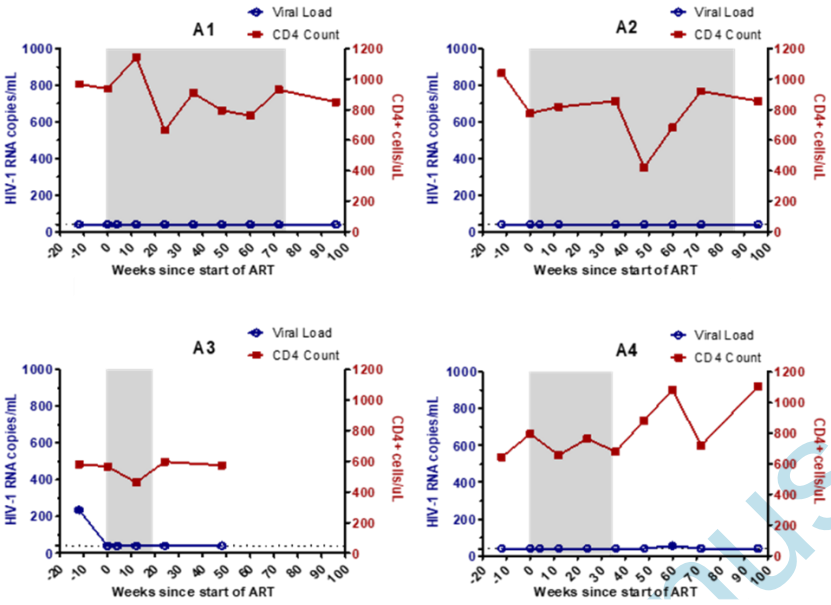


Figure 1. Evolution of CD4+ and CD8+ T-cell responses on antiretroviral therapy over time. Changes in T-cell responses over time (weeks on treatment) as measured by the percentage of T-cells positive for IFNγ, IL-2, TNFα, CD107a/b, and the combination IFNγ/TNFα/CD107. Medians and interquartile ranges are plotted. The number of participants contributing data to each time point are given along the x-axis. †P-values represent changes in each cytokine from pre-ART to 24-48 weeks on ART by repeated measures generalized estimation equations (GEE models).

A.



B.

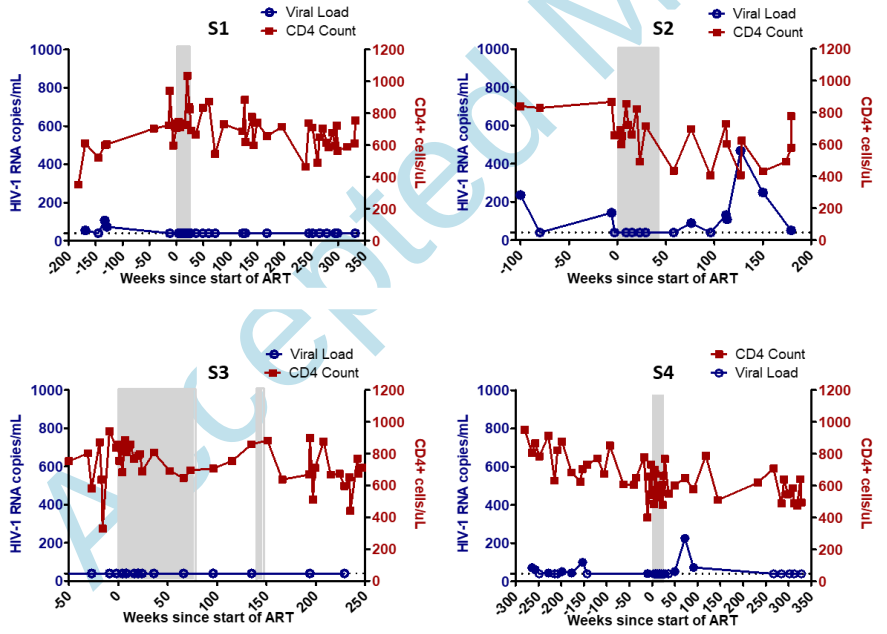


Figure 2. CD4+ T cell counts and plasma HIV-1 RNA levels over time (Abbott assay). CD4+ T cell counts and plasma HIV-RNA levels in 4 A5308 (A) and 4 SCOPE (B) participants who were monitored after stopping antiretroviral therapy (ART). Time since initiation of ART is given in weeks, and time on ART is marked in gray.