Acute HIV Treatment Reverses CD8 T Cell Activation, Exhaustion, and Apoptosis

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ABSTRACT

Background: Despite viral suppression with cART, persistent inflammation contributes to pathogenesis and morbidity. We hypothesized that treatment during acute HIV infection (AHI) with an integrase inhibitor based regimen would decrease cellular activation, exhaustion, and apoptosis compared with subjects treated in chronic infection.
Methods: Subjects identified in AHI (n= 18, Fiebig stages I-IV), were treated with tenofovir/emtricitabine and either raltegravir (RAL) or efavirenz (EFV). Matched chronically HIV infected subjects (CHI) (n=14) served as controls. Using flow cytometry, we measured surface expression of markers of activation (CD38), exhaustion (PD-1, CD160, CD244), senescence (CD57), pro-survival (Bcl-2) and apoptosis (cleaved-caspase-3) on CD8 T cells pre- and post-treatment. Comparisons were made within groups (Wilcoxon rank) and between groups (Mann-Whitney test), p< 0.05 denoted by *.
Results: Treatment in both AHI and CHI cohorts significantly reduced the frequency of activated CD38+ CD8 T cells (acute pre ART and virally suppressed (VS): 28% vs 6.3%,*, chronic 24% vs 17%,*), and, VS AHI had lower frequency compared with VS CHI (6.3% vs 17%,*). Treatment also reduced some markers of exhaustion, reducing the frequency of PD-1hi CD8 T cells in both groups (AHI 5.6% vs 2.3%,*, CHI 11% vs 7.1%,*), with greater reduction in VS AHI (VS AHI 2.3% vs VS CHI 7.1%,**). Treatment in both AHI and CHI reduced the frequency of apoptotic CD8+ T cells (CC-3hi/Bcl-2lo) (AHI 8.7% vs 2.9%,*,CHI 8.3% vs 5.2%,*), with greater reduction in VS AHI_(VS AHI 2.9% vs VS CHI 5.2%,*). Despite early treatment, viral suppression in AHI did not impact expression of CD160, CD57, or CD244. Enumeration of memory markers (naïve, central (CM) and effector memory (EM)) demonstrated that AHI treatment resulted in fewer CD8 T EM populations when compared to CHI (4.9% vs 32%,*), but did not impact naïve or CM.
There was a trend to faster time to undetectable VL in the RAL vs EFV arm. No differences were observed in immune parameters between RAL vs EFV groups. Conclusions: Treatment in AHI resulted in more subjects achieving normal CD4% (AHI 85% vs CHI 22%,*) and CD4/CD8 ratios of >1 (AHI 95% vs CHI 60%,*).
Treatment during acute HIV reverses some measures of immune inflammation, including CD8 T cell activation (CD38), exhaustion (PD-1), and apoptosis (CC-3hi/Bcl-2lo) and results in more robust immune recovery. These data reinforce that early identification and treatment are warranted to improve clinical outcomes.

Background

Despite ART, chronic HIV infection is associated with an immunopathology of chronic activation, immunosenescence, and accumulation of terminally differentiated T cells, contributing to an increased risk of morbidity and mortality. Early ART initiation can improve clinical markers including CD4 counts. However, there are conflicting reports on the effect of acute treatment on T cell activation. Furthermore, little is known about the effect of early treatment during acute infection on markers of T cell senescence, exhaustion, and apoptosis. Given that HIV immunopathogenesis is likely related to T cell activation, T cell dysfunction, exhaustion, and senescence, we sought to investigate these immune parameters along with clinical markers of disease progression in the setting of treatment during acute HIV infection (AHI) compared with treatment during chronic HIV infection (CHI).

Cohort / Methods

Patient Group	N	Plasma Viral Load (RNA copies/ml)			Absolute CD4 count (cells/µl)		
		Pre ART	On ART	Off ART	Pre ART	On ART	Off ART
Acute (RAL)	9	5,910-10,000,000	19-320	NA	251-959	399-1251	NA
Acute (EFV)	9	7,220-6,310,000	19-865	NA	245-573	336-903	NA
Chronic	14	NA	<50	1,630-409,120	NA	258-1025	12-964

Volunteers were recruited from the UAB HIV clinic. HIV diagnostics, CD4 T cell counts and viral loads were obtained from the patient record. Subjects with Acute HIV Infection (AHI) were Fiebig stage I-IV. PBMC were isolated from HIV+ individuals and cultured at a concentration of 1 x 10^6 cells/mL in 10% AB media at 37° C for 6 hours. Peptide stimulation was performed using optimized epitopes to HIV. Cells were washed and stained first with live/dead Fixable Dead Cell Stain followed by surface staining for anti-CD3, CD4, CD8, CD27, CCR-7, CD45RO, PD-1, CD244, CD160, CD38, and CD57. This was followed by intracellular staining for cleaved caspase 3 (Asp 175) (CC3) a common effector caspase to identify apoptotic cells and Bcl-2, an anti-apoptotic/pro-survival molecule. Data was collected on an LSRII Flow cytometer and analyzed using FlowJo software. Lymphocytes were gated on live, CD3+/CD8+ cells. Statistical analysis was performed using the Students T test and Mann Whitney U test.

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RESULTS

Treatment regimen & time to viral load control

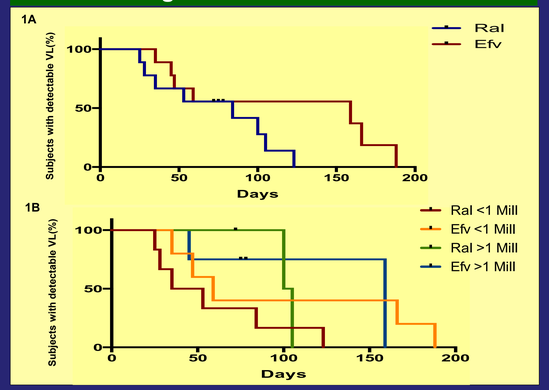


Figure 1. Treatment regimen and time to viral load control.
1A) Frequency of subjects with detectable viral load over time following treatment with raltegravir vs efavirenz based regimens.
1B) Frequency of patients with detectable viral load over time following treatment with raltegravir vs efavirenz based regimens, when initial viral loads are greater or less than 1 million copies / ml.
Kaplan-Meyer curves were compared by Mantel-Cox log-rank test, and tick marks represent patients who did not reach undetectable viral load. In each case, the raltegravir treated group reaches undetectable viral load more rapidly than the efavirenz treated group, but differences are not statistically significant.

Treatment during AHI results in more effective Immune Reconstitution

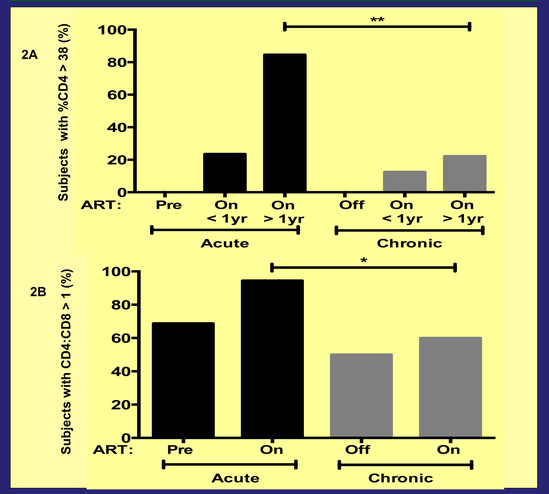


Figure 2. Effect of acute vs chronic treatment on CD4 recovery.
4A) CD4 T cell frequency (%CD4 > 38) was compared among acute and chronic subjects before ART and after short and long term ART treatment. A normalized CD4 frequency was defined as >38%.
4B) PBMC were stained for CD4 and CD8 and ratios were determined before and after ART treatment in acute and chronic infection. A normalized CD4 to CD8 ratio was defined as >1. Subjects who initiated ART during acute HIV infection achieved normalized CD4 frequencies and CD4 to CD8 ratios more frequently than those who initiated ART during chronic HIV infection. Data are compared using Fisher's exact test, *p<0.05, **p<0.01.

Treatment during AHI reverses some measures of CD8⁺ T cell activation, exhaustion, and apoptosis

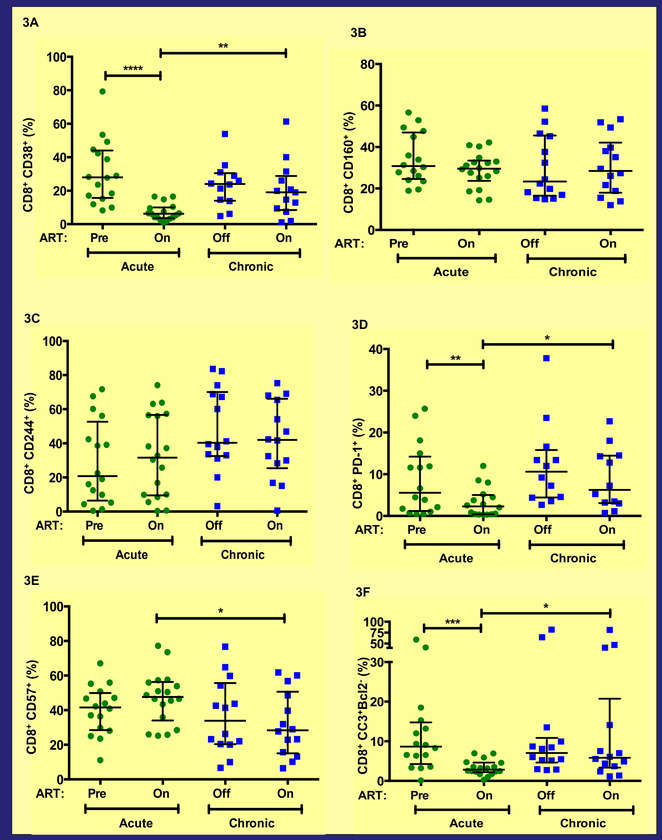


Figure 3.Effect of acute vs chronic treatment on CD8⁺ T cell activation, exhaustion, and apoptosis.
Using Flow cytometry, we measured markers of activation (Figure 3A- CD38, 3B- CD160, 3C, CD244), measures of exhaustion (Figure 3D- PD-1), measures of immunosenescence (Figure 3E- CD57) and measures of apoptosis and cell survival (Figure 3F- cleaved caspase -3 and Bcl-2) on CD8 T cells from subjects before and after treatment during AHI and CHI. Bars represent median +/- interquartile range. Data are compared within groups using two-tailed Wilcoxon signed rank test and between groups using two-tailed Mann-Whitney test.
*p< 0.05, **p< 0.01, ***p<0.001.

The frequency of activated CD8+ T cells, as measured by CD38, declines with treatment of HIV infection during acute and chronic HIV, however, the decline is greater when treatment occurs during AHI. We did not observe measurable differences in the frequency of CD160hi or CD244hi CD8 T cells with treatment in either group.
The frequency of PD-1hi CD8+ T cells, a measure of both activation and exhaustion, decreased with treatment during AHI and to a lesser degree with treatment during CHI. The decline was greater with treatment of AHI compared to treatment during CHI.
The frequency of immunosenescent CD8+ T cells, as measured by expression of CD57, did not decrease with treatment in either group.
The frequency of pro-apoptotic CD8+ T cells, as measured by CC-3hi/Bcl-lo, decreased with treatment during AHI but not CHI, and was significantly lower in subjects treated during AHI when compared to those treated during CHI.

Impact of Treatment on Memory Subsets

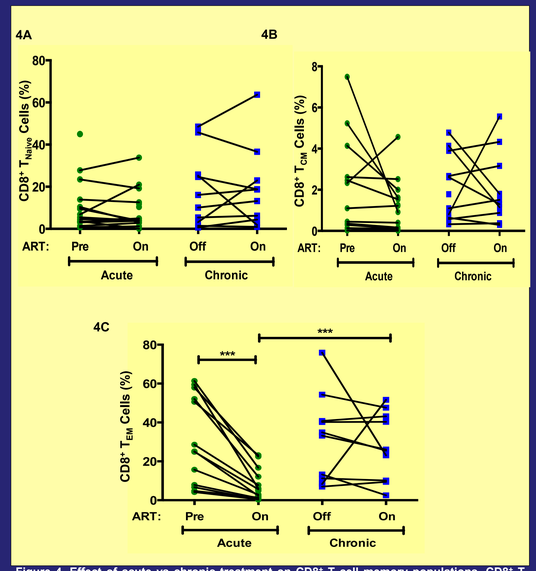


Figure 4. Effect of acute vs chronic treatment on CD8⁺ T cell memory populations. CD8⁺ T cells were stained for CCR7, CD27, and CD45Ro and classified as 3A) Naïve (CCR7+CD27+CD45Ro-); 3B) Central Memory (CCR7+CD27+CD45Ro+); and 3C) Effector Memory (CCR7-CD27+/-CD45Ro+). The frequency of effector memory CD8⁺ T cells decreased following treatment initiation during acute but not chronic infection and the frequency of effector memory CD8 T cells was lower after treatment in AHI compared to treatment in CHI. There is no change in other memory populations. Data are compared within groups using two-tailed Wilcoxon signed rank test and between groups using two-tailed Mann-Whitney test. ***p<0.001.

Conclusions

- Treatment during Acute HIV Infection (AHI) rapidly reduces viral load
  - We observed trends to more rapid viral control with Raltegravir compared to EFV based regimens
- Treatment during AHI results in greater immune reconstitution compared with CHI (CD4% > 38 and CD4:CD8 ratio > 1)
- Treatment during AHI reverses measures of activation (CD38), exhaustion (PD-1), and apoptosis (CC-3hi/Bcl-2lo) on CD8 T cells
- While treatment during chronic HIV improves some measures of immune function, we only observed small trends in the reversal of the measures of activation, exhaustion, and apoptosis in this cohort.
- In most cases, the degree of activation, exhaustion, and apoptosis were significantly lower in subjects treated in AHI compared to CHI.
- Persistence of activated, exhausted, and senescent CD8+ T cells contributes to immunopathogenesis related to inflammation in HIV
- This work adds to a growing body of literature supporting treatment early after infection to rapidly reduce viral load, reconstitute the immune system, and preserve immune function.