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Antiretroviral therapy initiated during acute HIV infection fails to prevent persistent T cell activation

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Abstract

Initiation of ART during acute HIV-1 infection may prevent persistent immune activation. We analyzed longitudinal CD38+HLA-DR+ CD8+ T cell percentages in 31 acutely infected individuals who started early (median 43 days since infection) and successful ART, and maintained viral suppression through 96 weeks. Pre-therapy a median of 72.6% CD8+ T cells were CD38+HLA-DR+, and while this decreased to 15.6% by 96 weeks, it remained substantially higher than seronegative controls (median 8.9%, p=0.008). Shorter time to suppression predicted lower activation at 96 weeks. These results support the hypothesis that very early events in HIV-1 pathogenesis may result in prolonged immune dysfunction.

Keywords

acute HIV infection; antiretroviral therapy; immune activation; viral dynamics; NNRTIs

INTRODUCTION

Interventions during acute HIV infection (AHI) may present a unique opportunity to alter the pathogenesis of the disease. In the natural history of HIV infection, early and complex virus-host interactions lead to chronic depletion and hyperactivation of T cells. Within
weeks of HIV acquisition, the host innate immune system responds with a cytokine storm that likely produces the symptoms of acute retroviral syndrome.\textsuperscript{1,2,3} CD4+ and CD8+ cells become activated. But for rare individuals, host immune responses reduce but fail to fully suppress viremia. During acute infection and in the absence of treatment, CD8+ cell activation reaches a steady state.\textsuperscript{4} And while the mechanisms by which immune activation contributes to immunodeficiency are not fully understood, an individual’s level of CD8+ cell activation independently predicts the rate at which HIV disease progresses.\textsuperscript{5}

Antiretroviral therapy (ART) reduces mortality in HIV-infected individuals through virologic suppression (VS), reconstitution of CD4+ cells, and reduction of T cell activation. Although ART reduces T cell activation, many persons with treated and suppressed chronic HIV infection have persistently elevated levels of activation.\textsuperscript{6,7} Persistent activation may slow the reconstitution of CD4+ cells and increase mortality both from AIDS and non-AIDS events.\textsuperscript{6} While the phenomenon of persistent immune activation is primarily described following chronic HIV infection (CHI), the activation outcomes of acutely infected and treated individuals are not well described.

While effective,\textsuperscript{8} the personal health benefits of starting ART during AHI are unproven.\textsuperscript{9,10} A short course of ART started during AHI reduces the symptoms of acute retroviral syndrome and may decrease the rate of CD4+ cell count decline after ART discontinuation.\textsuperscript{11} In addition, ART provided during AHI may reduce the viral set point.\textsuperscript{12,13} While the possible benefits of providing a short course of ART during AHI have been investigated, the impact of long term ART begun early after infection on immune activation is unknown.

In this study, we analyzed longitudinal dynamics of CD8+ cell activation in acutely infected adults treated with ART. We selected patients with the best possible treatment outcomes (those who started immediate ART, achieved VS, and at 96 weeks remained suppressed) in order to determine the efficacy of ART in preventing chronic persistent immune activation. We compared CD8+ cell activation levels of AHI patients to seronegative controls, and determined whether the timing of ART initiation or rapidity of VS were associated with levels of activation in the setting of effectively treated HIV-1.

**METHODS**

Adults diagnosed with AHI and linked to care at University of North Carolina at Chapel Hill (UNC), Duke University, or Emory University were enrolled in an open label, NNRTI- or PI-based ART treatment trial within 45 days of AHI diagnosis. We defined AHI by a negative or indeterminate enzyme immunoassay (EIA) or a negative HIV RNA test within 45 days of enrollment plus reproducibly detectable HIV RNA by amplification methods. All participants had blood sent for baseline HIV genotyping prior to ART initiation. We included only patients who achieved VS (HIV RNA ≤200 copies/mL) by 48 weeks and sustained suppression through 96 weeks. Patients were excluded if CD8+ activation was not measured pre-treatment and then serially through 96 weeks (±12 weeks).

At enrollment, acute retroviral syndrome (ARS) symptoms were recorded as previously described.\textsuperscript{8,14} We estimated the date of HIV infection as 14 days prior to the onset of symptoms consistent with ARS.\textsuperscript{3,15} The time (in days) from the estimated date of infection to AHI diagnosis and to ART initiation was calculated. Sexually transmitted infections (STIs) in the 8 weeks preceding HIV diagnosis were recorded.

Prior to ART and at weeks 12, 24, 48, 72, and 96 after therapy initiation, the HIV RNA level and the percent of CD8+ cells expressing CD38 and HLA-DR were measured. HIV RNA was quantified using the Roche Amplicor Monitor ultrasensitive assay or the Abbott
RealTime HIV-1 assay. The percent CD38+ HLA-DR+ CD8+ cells was measured on fresh blood samples collected in EDTA tubes. Activated CD8+ cells were identified using the following antibodies: αCD3-PerCP, αCD8 FITC, αHLA-DR-APC, and αCD38-PE. Antibody staining was evaluated using a 4-color Calibur flow cytometer instrument (Becton Dickinson, San Jose, CA). In the same laboratory, thirty HIV-seronegative volunteers (70% women and median age 32 years) provided fresh blood samples for measurement of CD8+ cell activation using the same methods. Beyond age and gender, the clinical characteristics of seronegative volunteers were not available. We defined normal CD8+ activation as less than or equal to the 95th percentile of the seronegative cohort’s distribution of activation levels.

Pearson’s Chi-squared test was used for bivariable analyses between categorical baseline characteristics and immune activation. The Wilcoxon rank-sum test was used to compare the percent activation between acutely HIV infected and seronegative participants and for continuous pre-therapy characteristics of the AHI cohort. We calculated the proportion of AHI patients whose T cell activation normalized by 96 weeks. Using multivariable linear regression with generalized estimating equations, we determined the impact of the timing of ART initiation and the rapidity of VS on immune activation. SAS version 9.2 (Cary, NC) and Stata 11 (College Station, TX) were used for analyses. The study was approved by the UNC and Duke Institutional Review Boards. All participants provided written informed consent.

RESULTS

Of 138 adults with AHI who initiated ART within 45 days of diagnosis since 1998, 31 met the criteria for analysis. We excluded 14 AHI patients without sustained suppression for 96 weeks, 67 without CD8+ activation markers measured longitudinally, and 26 who had not yet reached 96 weeks. Baseline demographic, virologic, and immunologic characteristics were similar between those included and excluded from the analysis (data not shown). Median age at therapy initiation was 32 years (range 19-64) and 29 patients (94%) were men (Table 1). At diagnosis, 97% reported at least one (median 6) ARS-associated symptom. One participant reported no symptoms of AHI and therefore, his estimated date of infection was not determined. In the 8 weeks preceding AHI diagnosis, 3 (10%) participants had a documented STI (genital herpes, chlamydia, or non-gonococcal urethritis).

At AHI diagnosis, 16 (52%) participants tested EIA negative, 9 (29%) tested EIA positive but western blot (WB) negative or indeterminate, and 6 (20%) tested EIA and WB positive with a negative or indeterminate test in the prior 30 days. Each patient had at least two pre-ART viral loads. The median observed peak HIV RNA level was $5.86 \log_{10}$ copies/ml (IQR 5.49-6.15). By the date of therapy initiation the median HIV RNA level had decreased to $5.27 \log_{10}$ copies/ml (IQR 4.50-5.77). The median CD4+ cell count nadir was 392 cells/mm$^3$, and 5 (16%) participants had a nadir CD4+ cell count less than 200 cells/mm$^3$.

The median time from acquisition to AHI diagnosis was 25 days (IQR 19-31) and from acquisition to ART initiation was 43 days (IQR 39-53). In this cohort, 28 (90%) patients received NNRTI-based regimens (co-formulated emtricitabine, tenofovir, and efavirenz or co-formulated emtricitabine and tenofovir plus nevirapine), 1 patient received a PI-based regimen ( stavudine, didanosine, and nelfinavir), and for 2 participants the regimen type was missing. We were not able to compare the effect of regimen type on immune activation. Three patients (10%) had transmitted drug resistance (TDR), but only 1 patient required a change of therapy. The median time to VS was 12 weeks (IQR 8-17).
Pre-therapy, the median percent CD38+HLA-DR+ CD8+ cells was 72.6 (IQR 22.6-92.6). CD8+ activation decreased substantially from baseline to week 12 and then more slowly during subsequent measurements (Figure 1). From weeks 48 to 96, the median activation of ART-treated AHI patients decreased by 3.8% (p=0.07). However, at 96 weeks, the median percent CD38+HLA-DR+ CD8+ cells remained higher in participants with ART-treated AHI versus the seronegative controls [15.6 (IQR 11.1-22.6) vs. 8.9 (IQR 5.8-14), p<0.01]. Approximately 40% of AHI patients failed to reach normal levels of activation by 96 weeks (Figure 1).

In bivariable analyses, log_{10} peak viral load, pre-treatment CD8+ cell activation, and time to VS were associated with CD8+ activation at 96 weeks (all p<0.05). Among both the AHI patients and seronegative controls, we did not find an association between age or gender and CD8+ activation. Recent STI diagnosis among AHI patients was also not associated with higher levels of immune activation. In multivariable analyses, shorter time from estimated date of infection to ART initiation was not associated with a reduction in CD8+ activation at 96 weeks (p=0.6). However, after adjustment for age, CD4+ cell nadir, and peak viral load, shorter time to VS predicted a reduction of CD8+ activation at 96 weeks (p=0.002). For every 1 month decrease in time to VS, CD8+ activation at 96 weeks decreased by 0.85%.

As part of a recently published study, four (13%) participants also underwent measurements of the size of the viral reservoir (at mean 92 weeks of ART). There was a positive but non-significant correlation between the log_{10}-transformed frequency of latently infected cells (reservoir size) and CD8+ activation at 96 weeks (Spearman’s rho=0.8, p=0.2), as well as a correlation between frequency of latently infected cells and low-level plasma viremia as measured by a single-copy assay.16

**DISCUSSION**

Among 31 acutely HIV infected patients with rapid linkage to care, early initiation of ART, and rapid and durable VS, CD8+ activation decreased substantially during the first 12 weeks of therapy and then decreased more slowly with increased duration of VS. Despite 2 years of suppressive ART, AHI patients had a higher CD8+ activation compared with seronegative controls, with 40% failing to reach normal levels. While not fully understood, immune activation appears to play a central role in HIV pathogenesis, and this study builds on the existing evidence in CHI that immune activation persists in many patients.6,7 Data from this study add to a discordant story regarding the immunologic benefits of starting ART during acute/early infection.4 During one ART interruption trial, patients who started ART during acute versus early HIV infection had no difference in activated CD8+ cells at 48 weeks.18 However, more recently patients who started ART earlier (median 75 days of infection) had lower levels of CD8+ cell activation versus those who started later (median 3.1 years).19 We found no association between the timing of ART initiation and CD8+ activation, possibly due to the narrow range (21-67 days) of time to treatment within our cohort. As our cohort consisted of patients with successful and durable VS, our results may not be representative of all AHI patients. Further, as CD8+ activation levels may vary depending on laboratory technique and whether fresh or previously frozen cells are used, our results on fresh cells are not directly comparable to other cohorts of patients receiving ART. Unfortunately, a direct comparison of CD8+ activation between acute and chronically infected patients at our center, which would have strengthened our findings, was not feasible.

One possible explanation for persistence of immune activation is the extensive and rapid depletion of gut associated lymphoid tissue (GALT) which may result in a state of chronic
hyperactivation and inadequate immune restoration.\textsuperscript{20,21} Persistently elevated levels of activated lymphocytes were found in the GI tract 3-7 years after suppressive ART was initiated during acute or early infection even while peripheral blood activation markers normalized.\textsuperscript{22} In our study, while only 60\% of patients by 96 weeks had reached a level within the range of uninfected subjects, activation decreased with increased duration of VS (Figure 1). This observation suggests that with time, further normalization of peripheral blood activation markers may occur. However, peripheral blood lymphocytes represent only a tiny fraction of all lymphocytes and GALT may be a better (but more logistically challenging) site for examination of the overall immunological status of HIV infected individuals.

While median levels of CD8+ activation remained higher than controls, we found that more rapid VS predicted reduced immune activation. Despite the very high viral load seen during AHI, the time to VS during ART may actually be shorter than seen with CHI.\textsuperscript{8} Time under the viremia curve in the setting of AHI has been shown to correlate with the size of the viral reservoir in resting CD4+ cells.\textsuperscript{16} A correlation between the frequency of latently infected cells and low-level plasma viremia as measured by a single-copy assay was also reported.\textsuperscript{16} Release of virus from this reservoir may produce low level viremia and immune activation.\textsuperscript{23} Therefore, limiting the reservoir size could reduce CD8+ activation. The correlation we identified, in 4 patients, between reservoir size and immune activation did not reach statistical significance. These preliminary observations deserve future study, and we suggest that therapy that rapidly suppresses viremia should be further examined in the setting of AHI.

The goal of this analysis was to investigate those individuals who had an optimal response to ART, in effect to evaluate a “best-case” scenario. Therefore, our cohort may not be representative of all AHI patients (due to our selection for durable VS). In addition, we had limited knowledge regarding the health of seronegative controls. The control group consisted of employees at a University hospital. Beyond age and gender, we unfortunately had no additional information regarding their medical histories. Pro-inflammatory co-infections (such as hepatitis B/C, CMV, and/or STIs) may have been less common among controls and could have produced the difference in CD8+ activation seen between ART-treated AHI patients and seronegative controls.

In the early stages of HIV infection, virus-host interactions result in steady states of HIV RNA and CD8+ activation. These parameters determine the rate at which HIV progresses, and may be associated with risk of death from non-AIDS-associated conditions.\textsuperscript{24} In this study, we found that initiation of ART during acute HIV-1 infection failed to prevent the persistence of immune activation at 96 weeks of suppressive therapy. These results suggest that the early pathogenesis of HIV disease leaves a long-lasting imprint on host immune function.

**Acknowledgments**

None

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Figure 1. CD8+ cell activation in AHI patients during 96 weeks of ART
Dynamics of CD8+ cell activation among 31 ART-treated acutely HIV infected individuals and comparison of 96 week activation levels to 30 seronegative controls.
Table 1
Baseline demographic and disease characteristics of 31 acutely HIV-infected individuals at initiation of ART.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>31</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>32 (19-64)</td>
</tr>
<tr>
<td>Male sex, N (%)</td>
<td>29 (94)</td>
</tr>
<tr>
<td>Race/ethnicity, N (%)</td>
<td></td>
</tr>
<tr>
<td>White/non-Hispanic</td>
<td>19 (61)</td>
</tr>
<tr>
<td>African-American</td>
<td>12 (39)</td>
</tr>
<tr>
<td>Sexual risk group, N (%)</td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>24 (77)</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Symptoms of ARS, median N (range)</td>
<td>6 (0-15)</td>
</tr>
<tr>
<td>Testing pattern at diagnosis, N (%)</td>
<td></td>
</tr>
<tr>
<td>EIA neg/NAAT pos</td>
<td>16 (52)</td>
</tr>
<tr>
<td>EIA pos/WB neg/ind</td>
<td>8 (26)</td>
</tr>
<tr>
<td>EIA pos/WB pos with neg/ind test past 30 days</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Nadir CD4+ cell count, median cells/mm³ (IQR)</td>
<td>392 (289-501)</td>
</tr>
<tr>
<td>Peak viral load, median log₁₀copies/ml (IQR)</td>
<td>5.86 (5.49-6.15)</td>
</tr>
<tr>
<td>CD38+HLA-DR+CD8+ cells, median percent (IQR)</td>
<td>72.6 (22.6-92.6)</td>
</tr>
</tbody>
</table>

ARS-acute retroviral syndrome; EIA-enzyme immunoassay; NAAT-nucleic acid amplification test; WB-western blot