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CD8+ T-cell Activation in HIV-1-Infected Patients Experiencing Transient Low-level Viremia During Antiretroviral Therapy

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Abstract

Transient low-level viremia of 50–400 HIV RNA copies/mL (TLLV) is common during antiretroviral therapy, but its pathogenesis, consequences and optimal management are unclear. Heightened immune activation is associated with detrimental outcomes, including impaired CD4+ T-cell reconstitution. Using CD38/HLA-DR expression on CD8+ T-cells measured in two large studies, we determined associations between TLLV and immune activation levels before, during, and after TLLV. We found that TLLV does not significantly change CD8+ T-cell activation, and that higher CD8+ T-cell activation during viral suppression <50 copies/mL is associated with a modest increase in the risk of a subsequent TLLV.

Keywords

low-level; viremia; blip; immune; activation; CD8+

INTRODUCTION

Transient low-level viremia of 50–400 copies/mL (TLLV) is common after plasma HIV RNA suppression to <50 copies/mL during antiretroviral therapy (ART) [1–4], but its pathogenesis, consequences and optimal management are unclear. Occurrence of TLLV is

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Conflicts of Interest

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sometimes linked to lapses in adherence [5]. It is uncertain whether TLLV reflects release of archived viruses from reservoirs and/or bursts of viral replication. Random HIV RNA variation around a mean value <50 copies/mL [3] and laboratory assay artifacts [6] are other potential explanations. Detection of HIV RNA below 50 copies/mL may also be a risk factor for TLLV since detection of HIV RNA below 50 copies/mL correlates with subsequent virologic rebound [7]. Compared to transient viremia above 400 copies/mL, TLLV is less likely to increase the risk of virologic failure or antiretroviral drug resistance [1, 3, 4, 8], although some studies suggest TLLV may be important clinically [7, 9, 10].

The dominant source of immune activation in untreated HIV infection is antigenic stimulation, whether by HIV, co-pathogens, or products of bacterial translocation from the gut [11–13]. Markers of immune activation decline during ART; however, the levels remain abnormally elevated after ART-mediated viral suppression [14–16]. It is unknown whether TLLV causes recrudescence of HIV antigenic stimulation and immune activation, or whether elevated immune activation causes TLLV. Understanding these issues is important because heightened immune activation has been linked to detrimental outcomes such as blunted CD4+ T-cell recovery [15, 17]. If TLLV elevates immune activation, one would expect that an episode of TLLV would precede an increase in activation. If, alternatively, heightened immune activation triggers transient release of HIV from reservoirs and results in TLLV, then an episode of TLLV would be preceded by an increase in immune activation.

To dissect these issues, we determined within-subject changes in immune activation, as measured by CD38/HLA-DR expression on CD8+ T-cells, before, during and after TLLV. We also conducted between-groups analyses, comparing immune activation levels during viral suppression <50 copies/mL between subjects who subsequently experienced TLLV versus those who maintained HIV RNA <50 copies/mL. In an additional between-groups analysis, we explored whether having detectable HIV RNA below 50 copies/mL at a pre-TLLV time point is predictive of a subsequent TLLV

METHODS

We analyzed data from AIDS Clinical Trials Group (ACTG) study A384, a randomized trial in treatment-naïve HIV-infected patients [18, 19] and ACTG Longitudinal Linked Randomized Trials (ALLRT), an observational cohort of HIV-infected patients who initiated ART in randomized clinical trials [20]. CD38/HLA-DR expression on CD8+ T-cells was measured on fresh PBMC samples using ACTG consensus methods every 16–24 weeks in 623 subjects in ACTG A384 [16, 17] and in 2391 subjects during 2000–2003 in ALLRT (every 16 weeks 2000–2002, every 48 weeks 2002–2003). HIV RNA quantification in ACTG A384 and ALLRT was performed using the Roche Amplicor assay v1.0 or v1.5 (Roche Diagnostic Systems, Branchburg, New Jersey, USA). HIV RNA from 1997–2012 were examined for TLLV (measured every 8 weeks after week 24 in A384; every 16 weeks in ALLRT through 2006, then twice every 48 weeks). Samples with <50 HIV RNA copies/mL were qualitatively sub-classified into HIV RNA undetected or detected based on optical density readings <0.2 or ≥0.2 in the Roche assay [21]. Institutional Review Boards at participating sites approved the protocols; subjects provided written informed consent.

We defined TLLV as an isolated HIV RNA 50–400 copies/mL that was preceded and followed by values <50 copies/mL with no more than 6 months between measurements and no change in the ART regimen. We included subjects who 1) experienced TLLV after at least 6 months of HIV RNA <50 copies/mL, and 2) had immune activation data available before, during and after an episode of TLLV (Aim 1), and at one timepoint of interest for Aim 2 (pre-TLLV). In subjects who had more than one episode of TLLV, we only analyzed the first occurrence (along with immune activation data around the time of that event).

For Aim 1 (longitudinal within-subject analysis), each eligible subject served as his/her own control. CD8+ T-cell activation was compared between the TLLV time point and the pre- and post-TLLV time points using repeated measures analysis (generalized estimating equations with identity link). In supplemental analyses, we compared just the pre-TLLV and TLLV time points, and the TLLV and post-TLLV time points. Sensitivity analyses examined the subset of subjects whose TLLV amplitudes were 50–200 copies/mL vs. 201–400 copies/mL. Variability (standard deviation, SD) in the change in CD8+ T-cell activation over short time frames during viral suppression was estimated to be 8.5% (percentage-points) over 48 weeks in ACTG 384. Using SD=8.5%, a sample size of 66 subjects was estimated to provide statistical power >80% to detect a 3.0 percentage-point within-subject increase in immune activation.

For Aim 2 (cross-sectional between-groups analysis), we employed a case-control design and conditional logistic regression to compare CD8+ T-cell activation during viral suppression (HIV RNA <50 copies/mL) between subjects who subsequently experienced TLLV (cases) and those whose HIV RNA remained <50 copies/mL (controls). Case and control subjects were matched by parent study, sex, age (± 5 years), duration of HIV RNA suppression (± 6 months) and duration of ART (± 6 months).

Finally, in an exploratory analysis we focused on subjects with information on whether HIV RNA was qualitatively detected or undetected when HIV RNA was <50 copies/mL; conditional logistic regression evaluated whether detection versus non-detection of HIV RNA at the pre-TLLV time point with HIV RNA <50 copies/mL was predictive of a subsequent TLLV.

RESULTS

Population with TLLV in A384 and ALLRT

A total of 1861/5042 (37%) subjects experienced TLLV. For those with TLLV, there was a median of 6.0 person-years of follow-up and a median of 31 HIV RNA measurements per subject.

No significant within-subject change in CD8+ T-cell activation pre-, during and post-TLLV

There were 64 subjects who had TLLV and CD8+ T cell activation data available at the pre-TLLV, TLLV and post-TLLV time points; these subjects formed the population for the within-subject longitudinal analysis. They were 88% male with a median age of 39 years pre-TLLV. The median HIV RNA during TLLV was 94 (range, 50–400) copies/mL. The median (25th–75th percentiles) CD4+ T-cell count prior to TLLV was 539 (348–759) cells/mm³. Median (25th–75th) CD8+ T-cell activation before, during and after TLLV was 21% (10–33), 22% (12–33%), and 20% (12–31%), respectively. The pre-TLLV time point was a median (25th–75th) of 3.7 (3.4–3.9) months prior to the TLLV event, and the post-TLLV time point was 3.7 (3.6–4.0) months after the TLLV event. We did not detect a significant association between TLLV and CD8+ T-cell activation (95% CI: –1.4, 1.6 percentage-points, comparing pre- and post-TLLV to the TLLV time point; $p=0.88$), nor in CD8+ T-cell activation changes between the pre-TLLV and TLLV timepoints ($p=0.65$) or between the TLLV and post-TLLV timepoints ($p=0.86$). Results were similar in a sensitivity analysis restricted to 58 subjects with HIV RNA of 50–200 copies/mL at TLLV (95% CI: –1.8, 1.5 percentage-points; $p=0.84$).

Higher CD8+ T-cell activation during viral suppression is associated with increased risk of subsequent TLLV

In a cross-sectional between-groups analysis to examine whether CD8+ T-cell activation was associated with a subsequent episode of TLLV, cases were 123 subjects with subsequent TLLV (89% male; median age 42 years; median CD4 count=547 cells/mm³; median CD8+ T-cell activation=20%). CD8+ T-cell activation was measured a median (25th–75th) 1.9 (1.8–3.5) months prior to TLLV. There were 629 matched controls who maintained HIV RNA <50 copies/mL (88% male; median age 40 years; median CD4 count=490 cells/mm³; median CD8+ T-cell activation=18%). Figure 1 shows slightly higher CD8+ T activation in cases vs. controls. CD8+ T-cell activation at the time of viral suppression was significantly associated with increased risk of a subsequent TLLV (odds ratio=1.19 per 10 percentage points higher CD8+ T-cell activation; 95% CI: 1.01, 1.41; p=0.034). Neither CD4 cell count at the time of viral suppression nor nadir CD4 count was associated with risk of TLLV (p=0.20 and 0.27, respectively).

We conducted a similar case-control analysis using 118 case and 569 control subjects with information on whether HIV RNA was qualitatively detected when HIV RNA was <50 copies/mL. HIV RNA was detected in 10.2% of samples with <50 HIV RNA copies/mL. A trend towards increased risk of TLLV in the group with detected HIV RNA was found, but this did not reach significance (odds ratio=1.79; 95% CI: 0.96, 3.35, p=0.07).

DISCUSSION

We investigated associations between TLLV and immune activation using CD38/HLA-DR expression on CD8+ T-cells measured as part of two ACTG studies. In a longitudinal within-subject analysis, with each patient serving as his/her own control, we found no significant change in CD8+ T-cell activation during TLLV, consistent with results of a smaller study (N=15) that used a broader definition of low-level viremia (50–1000 HIV RNA copies/mL) [22]. However another study reported increased T-cell activation during transient viremia above 200 copies/mL, although no association was found when viremia was under 200 copies/mL [23]. In cross-sectional between-groups case-control analyses, higher CD8+ T-cell activation during viral suppression (HIV RNA <50 copies/mL) was associated with an increased risk of a subsequent TLLV, though this effect appears to be of limited magnitude.

The clinical ramifications of TLLV are unknown and most clinicians manage these episodes with adherence reinforcement. There have been signals, however, that TLLV may not be innocuous. Each episode lasts approximately 22 days [2] and increased TLLV frequency is associated with slower decay of latently infected cells [24]. Since heightened immune activation during ART may be detrimental, the absence of a significant increase in immune activation with TLLV in our study provides reassurance that TLLV is unlikely to cause clinical harm through this mechanism.

Although we found an association between higher CD8+ T-cell activation during viral suppression and increased risk of subsequent TLLV, there is no proof that higher immune activation is the cause of TLLV. Another potential explanation for the observed association is that increased CD8+ T-cell activation is related to as yet unidentified factors that drive TLLV. Alternatively, CD8+ T-cell activation and TLLV may be driven by common factors, such as incomplete adherence or larger virus reservoirs. \

In an exploratory analysis, we found a trend towards an association between qualitative detection of HIV RNA below 50 copies/mL and subsequent TLLV, but this did not reach statistical significance, perhaps due to small sample size. The fact that only 10% of subjects

with HIV RNA <50 copies/mL in our study had detectable HIV RNA raises questions about the sensitivity of the test; approximately two-thirds have detectable virus using single-copy assays [25]. Other studies have reported a relationship between HIV detection below 50 copies/mL and subsequent confirmed viral rebound [7, 9]

Our study has a number of limitations. First, some antiretroviral drugs used in ALLRT and A384 (such as unboosted protease inhibitors) are no longer recommended due partly to inferior virologic efficacy. We addressed this by focusing on patients who attained HIV RNA <50 copies/mL. Second, only one marker of immune activation (i.e., CD38/HLA-DR expression on CD8+ T-cells) was evaluated, constraining on our ability to address the full breath of immune activation and inflammation associated with HIV and ART. Potential co-infections on which we lacked information (such as herpesviruses) may have confounded our within-patient analysis. Hypothetically, co-infections can trigger immune activation, increase target T-cell availability and cause transient viremia [26]. The follow-up time points were over three months after the TLLV episodes and it is possible for an increase in T-cell activation due to the TLLV to have occurred sooner and waned by the follow-up time point. Finally, the relatively small number of patients in the analysis may limit the generalizability of our results.

In conclusion, TLLV during suppressive ART (HIV RNA <50 copies/mL) is unlikely to worsen CD8+ T-cell activation while higher CD8+ T-cell activation during viral suppression is associated with a modest increase in the risk of a subsequent TLLV. Future studies should further characterize the association between CD8+ T-cell activation milieu during viral suppression and subsequent TLLV, and probe the role of potential non-immunologic precursors of TLLV including residual viremia measured with single copy HIV RNA assay.

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Figure 1. CD8+ T-cell activation for 123 cases with subsequent transient low-level viremia and 629 matched controls who maintained HIV RNA <50 copies/mL. Horizontal bars display medians.