Impact of $CD8^+$ T-cell activation on $CD4^+$ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy

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Objectives: To assess whether T-cell activation independently predicts the extent of $CD4^+$ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy (ART).

Design: Prospective cohort study.

Methods: HIV-infected adults starting ART and achieving a plasma HIV RNA level (VL) less than 400 copies/ml by month 6 were sampled from the Uganda AIDS Rural Treatment Outcomes (UARTO) cohort in Mbarara, Uganda. CD4 count, VL, and the percentage-activated ($CD38^+HLA\text{-}DR^+$) T cells were measured every 3 months.

Results: Of 451 HIV-infected Ugandans starting ART, most were women (70%) with median pre-ART values: age, 34 years; CD4 count, 135 cells/μl; and VL, 5.1 $\log_{10}$ copies/ml. Of these, 93% achieved a VL less than 400 copies/ml by month 6 and were followed for a median of 24 months, with 8% lost to follow-up at 3 years. Higher pre-ART $CD8^+$ T-cell activation was associated with diminished CD4 recovery after year 1, after adjustment for pre-ART CD4 count, VL, and sex ($P = 0.017$). Thirty-four participants died, 15 after month 6. Each 10% point increase in activated $CD8^+$ T cells at month 6 of suppressive ART was associated with a 1.6-fold increased hazard of subsequent death after adjusting for pretherapy CD4 count ($P = 0.048$).

Conclusions: Higher pre-ART $CD8^+$ T-cell activation independently predicts slower $CD4^+$ T-cell recovery and higher persistent $CD8^+$ T-cell activation during ART-mediated viral suppression independently predicts increased mortality among HIV-infected Ugandans. Novel therapeutic strategies aimed at preventing or reversing immune activation during ART are needed in this setting.

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Introduction

Generalized immune activation is a hallmark of HIV infection and is established as an independent predictor of morbidity and mortality in the absence of antiretroviral therapy (ART) [1–6]. We and others have further demonstrated that, although generalized T-cell activation (assessed by the expression of CD38 and human leukocyte

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antigen-DR (HLA-DR) on $CD8^+$ T cells) declines during suppressive ART, it persists at abnormal levels in the majority of HIV-infected individuals despite years of viral suppression and is associated with poor $CD4^+$ T-cell recovery [7–11]. Soluble markers of inflammation also remain abnormally elevated during suppressive ART [12,13] and independently predict subsequent mortality and cardiovascular disease [14]. Thus, preventing the persistent inflammatory consequences of HIV infection during suppressive ART has emerged as a major challenge for the modern treatment era.

However, no study to date has linked cellular immune activation markers during treatment-mediated viral suppression to clinical outcomes. This is an important point because cellular markers of immune activation are increasingly being used as primary outcomes for pilot clinical trials of immune-based therapies in HIV infection given their better reproducibility and responsiveness than soluble inflammatory markers [15–18]. Furthermore, nearly all of the research evaluating the impact of immune activation on treatment-mediated immune recovery has been conducted in resource-rich settings and it remains unclear whether immune activation is a major determinant of $CD4^+$ T-cell recovery in resource-limited settings, in which the vast majority of treated HIV-infected individuals now live. Conceivably, differences in host genetics, viral clade, and prevalent coinfections (i.e., tuberculosis, malaria, helminthes, and so on) could alter the relationship between immune activation and clinical outcomes in this setting. To address these issues, we measured T-cell activation levels in a cohort of HIV-infected Ugandans starting their first ART regimen both before and during the first year of treatment-mediated viral suppression and assessed its association with subsequent $CD4^+$ T-cell recovery and mortality in this setting.

Methods

Participants

Ugandan participants were sampled from Uganda AIDS Rural Treatment Outcomes (UARTO), a cohort of 500 HIV-infected individuals starting their first nonnucleoside reverse transcriptase inhibitor-based combination ART regimen at a University clinic in Mbarara with a mostly rural catchment area in western Uganda. Study visits are performed every three months, which include extensive interviews, CD4 counts and plasma HIV RNA levels, and biological specimen archiving. During the first year of therapy, T-cell activation is measured on fresh specimens every 3 months.

Laboratory measurements

Plasma HIV RNA levels were first assessed using the Roche Amplicor HIV Monitor 1.5 test (Roche, Branchburg, New Jersey, USA; dynamic range 400–750 000 copies/ml). In April, 2007, this assay was replaced by the Roche Cobas Taqman HIV-1 test v1.0 (Roche; dynamic range 48–10 000 000 copies/ml).

T-cell activation

Freshly isolated peripheral blood was analyzed for T-cell activation using a Becton Dickinson FACSCalibur in the Cao laboratory at the Joint Clinical Research Center in Kampala. Whole blood was stained with fluorochrome-conjugated monoclonal antibodies: CD8-PerCP-Cy5.5, HLA-DR-FITC, CD38-PE and CD3-APC (BD Biosciences, San Jose, California, USA). Analysis was performed with FlowJo software v6.3.3 (Treestar, Ashland, Oregon, USA). T-cell activation was assessed by the frequency of $CD4^+$ and $CD8^+$ T cells that coexpressed CD38 and HLA-DR. Preset gating was applied to all samples and was based on the expression of activation markers in HIV-seronegative Ugandans [19]. A median of 42 000 [interquartile range (IQR): 30 000–54 000] $CD8^+$ T-cell and 14 000 (9000–20 000) $CD4^+$ T-cell events were acquired for all analyses.

Statistical methods

For the cross-sectional comparisons, continuous variables were compared between groups with Wilcoxon ranksum tests. Dichotomous variables were compared between groups with χ^2 and Fisher's exact tests. Relationships between continuous variables were assessed with Spearman's rank order correlation coefficients. Adjusted differences between groups were assessed with linear regression, calculating standard errors with heteroskedasticity-consistent covariance matrix estimators and log-transforming outcomes when necessary to satisfy model assumptions.

The rate and predictors of $CD4^+$ T-cell recovery among UARTO participants achieving a plasma HIV RNA level less than 400 copies/ml by month 6 of ART were assessed with linear mixed models (PROC MIXED in SAS) with square-root transformation of $CD4^+$ T-cell count to satisfy model assumptions. A three-piece segmented linear model (0–3 months, 3–12 months, and >12 months) was used to allow for changing slopes of $CD4^+$ T-cell recovery over time. Observations were censored at the time of a subsequent plasma HIV RNA level more than 1000 copies/ml. Baseline predictors of the rate of $CD4^+$ T-cell recovery in each segment were assessed with time-by-interaction terms. Factors associated with the rate of early or late $CD4^+$ T-cell recovery in unadjusted analyses ($P < 0.10$) were included as potential confounders in multivariable models. For interpretability, predicted values were back-transformed into the native scale for graphical interpretation [20]. Predictors of mortality were assessed with Kaplan–Meier methods and Cox proportional hazards models for adjusted analyses.

Table 1. Characteristics of untreated HIV-infected Ugandans initiating first antiretroviral therapy regimen.

Characteristic	Median (IQR) $N = 451$
Age, years	34 (29–39)
Female gender, No. (%)	316 (70)
$CD4^+$ T-cell count, cells/μl	135 (76–203)
Plasma HIV RNA level, $\log_{10}$ copies/ml	5.1 (4.5–5.5)
% $CD38^+$ HLA-DR^+ $CD8^+$ T cells	67 (56–76)
% $CD38^+$ HLA-DR^+ $CD4^+$ T cells	32 (22–45)

HLA-DR, human leukocyte antigen-DR; IQR, interquartile range.

Results

Characteristics of participants prior to initiating antiretroviral therapy

Among the 451 treatment-naive HIV-infected Ugandans contributing to these analyses, 70% were women, median age was 34 (IQR: 29–39) years, median pretherapy $CD4^+$ T-cell count was 135 (IQR: 76–203) cells/μl, and median plasma HIV RNA level was 5.1 (IQR: 4.5–5.5) $\log_{10}$ copies/ml (Table 1). Prior to starting ART, the median percentage activated ($CD38^+HLA\text{-}DR^+$) $CD8^+$ T cells was 67% (IQR: 56%–76%) and the median percentage activated $CD4^+$ T cells was 32% (IQR: 22%–45%). Higher pre-ART plasma HIV RNA levels were associated with both higher $CD8^+$ T-cell activation (ρ: 0.12, $P = 0.011$) and $CD4^+$ T-cell activation levels (ρ: 0.29, $P < 0.001$). Although pre-ART $CD4^+$ T-cell activation levels were associated with lower $CD4^+$ T-cell counts (ρ: −0.51, $P < 0.001$), there was no evidence for an association between pre-ART $CD8^+$ T-cell activation levels and pre-ART $CD4^+$ T-cell counts (ρ: −0.05, $P = 0.30$).

Relationship between T-cell activation and subsequent $CD4^+$ T-cell count recovery in antiretroviral therapy-suppressed Ugandans

Of the 451 Ugandans initiating ART, 93% [95% confidence interval (CI): 90–95%] achieved a plasma HIV RNA level less than 400 copies/ml by month 6 of therapy and were included in the analyses of $CD4^+$ T-cell recovery, censoring observations for a plasma HIV RNA level more than 1000 copies/ml. Median duration of follow-up prior to censoring was 24 months (IQR: 14–33 months) and only 8% were lost to follow-up at 3 years. Because the predictors of early (<3 months) and late (>12 months) $CD4^+$ T-cell recovery differ [21], we assessed predictors of $CD4^+$ T-cell recovery across three time segments using linear mixed models (0–3 months, 3–12 months, and >12 months). Although there was no evidence for a relationship between pretherapy $CD4^+$ T-cell activation or age and either early or late $CD4^+$ T-cell recovery in unadjusted analyses ($P > 0.51$ for all), pretherapy levels of $CD8^+$ T-cell activation, $CD4^+$ T-cell count, plasma HIV RNA level, and sex were all associated with the rate of either early or late $CD4^+$ T-cell recovery in unadjusted models ($P < 0.10$, Table 2). After adjustment for sex, pretreatment $CD4^+$ T-cell count, and plasma HIV RNA level, each 10% point increase in pretherapy $CD8^+$ T-cell activation was associated with a mean 0.03 fewer square root-transformed $CD4^+$ T cells/μl gained per month during months 3–12 ($P = 0.037$) and a mean 0.02 fewer square root-transformed $CD4^+$ T cells/μl gained per month after month 12 ($P = 0.017$). The independent effect of pretreatment $CD8^+$ T-cell activation on subsequent $CD4^+$ T-cell recovery can be visualized by plotting predicted $CD4^+$ T-cell count changes in two prototypic female participants, each with

Table 2. Predictors of $CD4^+$ T-cell recovery among HIV-infected Ugandans receiving suppressive antiretroviral therapy.

		Unadjusted		Adjusted	
Characteristic	Time segment (months)	Mean Δ in square root CD4 count (cells/μl)	*P* Value	Mean Δ in square root CD4 count (cells/μl)	*P* Value
Pretherapy $CD8^+$ T-cell activation, per 10% point increase	–	0.09	0.82	0.06	0.57
Pretherapy plasma HIV RNA level, per $\log_{10}$ copy/ml increase	–	−2.6	0.001	0.10	0.69
Pretherapy $CD4^+$ T-cell count, per 100 cell/μl increase	–	5.4	<0.001	4.0	<0.001
Female gender	–	1.89	0.13	0.01	0.97
	0–3	1.02	<0.001	0.96	<0.001
Per month	3–12	0.15	<0.001	0.14	<0.001
	12 onward	0.17	<0.001	0.12	<0.001
Pretherapy $CD8^+$ T-cell activation × month	0–3	−0.07	0.59	−0.02	0.57
	3–12	−0.02	0.25	−0.03	0.037
	12 onward	−0.01	0.09	−0.02	0.017
Pretherapy plasma HIV RNA level × month	0–3	0.68	0.009	0.28	0.002
	3–12	−0.04	0.15	−0.05	0.098
	12 onward	0.09	0.58	0.03	0.10
Pretherapy $CD4^+$ T-cell count × month	0–3	−1.0	<0.001	−0.4	<0.001
	3–12	−0.004	0.83	−0.04	0.050
	12 onward	0.008	0.46	0.03	0.034
Female gender × month	0–3	−0.38	0.38	0.32	0.012
	3–12	0.07	0.12	0.02	0.64
	12 onward	0.05	0.05	0.08	0.010

pretreatment $CD4^+$ T-cell counts and plasma HIV RNA levels set to median values, but who have different pretreatment $CD8^+$ T-cell activation levels set to the 15th (51%) and 85th (81%) percentile values (Fig. 1a). This adjusted difference in pretreatment $CD8^+$ T-cell activation is associated with a difference of 109 $CD4^+$ T cells/μl gained by year 3, and is comparable if not greater than the independent effect of pretreatment plasma HIV RNA levels (Fig. 1b), $CD4^+$ T-cell count (Fig. 1c), and sex (Fig. 1d) on subsequent $CD4^+$ T-cell recovery.

We next assessed the relationship between T-cell activation during treatment-mediated viral suppression and subsequent $CD4^+$ T-cell recovery among participants with available T-cell activation data at month 6 ($n = 267$) and month 12 ($n = 216$). After adjustment for pretherapy plasma HIV RNA level, pretherapy and month 6 $CD4^+$ T-cell count, and sex, there was no evidence for a relationship between the frequency of activated $CD8^+$ T cells at month 6 of suppressive ART and the rate of subsequent $CD4^+$ T-cell recovery ($P = 0.14$). However, higher frequencies of activated $CD8^+$ T cells at month 12 tended to predict a diminished rate of subsequent $CD4^+$ T-cell recovery after adjustment for pretherapy plasma HIV RNA level, pretherapy and month 12 $CD4^+$ T-cell count, and sex ($P = 0.075$, Supplementary Table 1, http://links.lww.com/QAD/A181).

Changes in T-cell activation during suppressive antiretroviral therapy

Changes in the percentage-activated $CD8^+$ and $CD4^+$ T cells were assessed every 12 weeks through week 48 among Ugandans achieving a plasma HIV RNA level less than 400 copies/ml by month 6, censoring observations for subsequent plasma HIV RNA levels more than 1000 copies/ml. $CD8^+$ T-cell activation declined more rapidly from baseline to month 6 (mean 3.8% per month, $P < 0.001$) than from month 6–12 (mean 1% per month, $P < 0.001$, P for interaction $= 0.001$, Fig. 2a). Although the mean rate of decline in $CD8^+$ T-cell activation after month 6 was similar regardless of pretherapy $CD8^+$ T-cell activation level (P for interaction $= 0.68$), the rate of decline in the first 6 months was more rapid for participants with higher pretherapy $CD8^+$ T-cell activation levels (P for interaction $= 0.001$). Despite early differences in the rate of decline in $CD8^+$ T-cell activation, those with higher pretherapy $CD8^+$ T-cell activation levels continued to have higher $CD8^+$

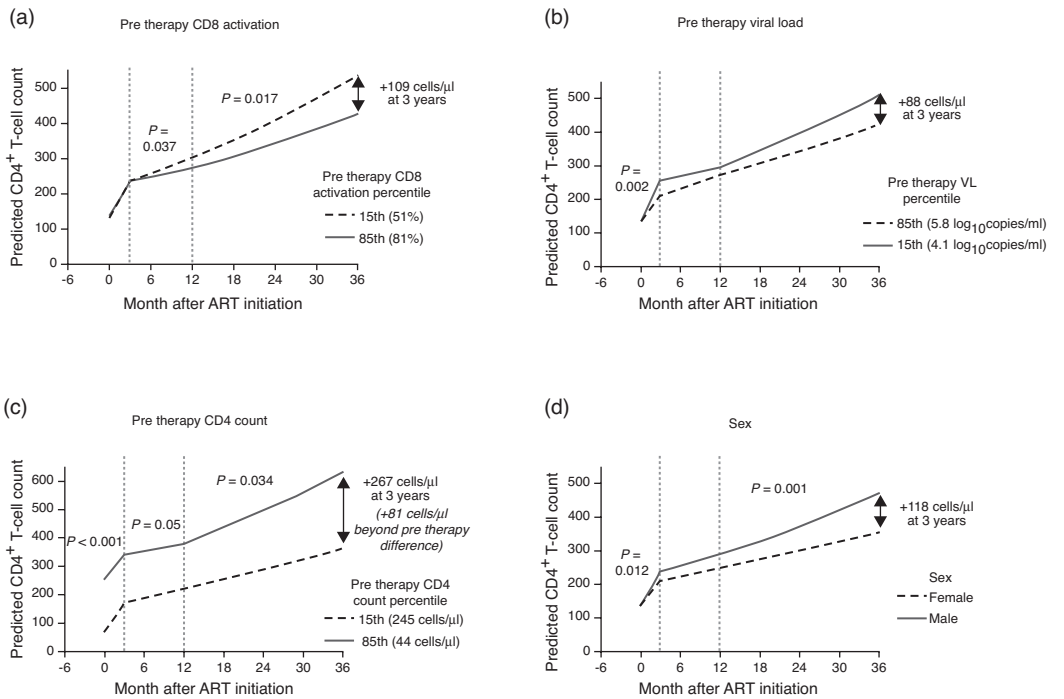

Fig. 1. Independent predictors of $CD4^+$ T-cell recovery in HIV-infected Ugandans maintaining treatment-mediated viral suppression. Pretherapy predictors of the rate of $CD4^+$ T-cell recovery were assessed in HIV-infected Ugandans with multivariable linear mixed models across 3 time segments. The plots depict the estimated mean $CD4^+$ T-cell changes for the 15th and 85th percentile values of each predictor in the multivariable model when setting all other baseline covariates in Table 2 to median values. P values test whether the slope of $CD4^+$ T-cell recovery varies according to the predictor of interest within each spline segment and the mean adjusted difference in $CD4^+$ T-cell count at month 36 is reported for each predictor. The independent effects on subsequent $CD4^+$ T-cell recovery are depicted for pre-treatment $CD8^+$ T-cell activation (a), plasma HIV RNA level (b), $CD4^+$ T-cell count (c), and gender (d). ART, antiretroviral therapy; VL, plasma HIV RNA level.

T-cell activation levels at month 12 (ρ: 0.43, $P < 0.001$, Fig. 2b). Higher month 12 $CD8^+$ T-cell activation was also associated with lower pretreatment $CD4^+$ T-cell counts (ρ:−0.25, $P < 0.001$) and with higher pretherapy plasma HIV RNA levels (ρ: 0.16, $P = 0.020$). In a multivariable model, higher month 12 T-cell activation levels were predicted by both lower pretreatment $CD4^+$ T-cell count ($P = 0.006$) and higher pretreatment $CD8^+$ T-cell activation ($P < 0.001$), but no longer by pretreatment plasma HIV RNA levels ($P = 0.67$).

Similar biphasic declines were observed in $CD4^+$ T-cell activation levels. Higher month 12 $CD4^+$ T-cell activation was also predicted by higher pretherapy $CD4^+$ T-cell activation (ρ: 0.57, $P < 0.001$), higher pretherapy plasma HIV RNA levels (ρ: 0.21, $P = 0.002$), and lower pretherapy $CD4^+$ T-cell counts (ρ: −0.44, $P < 0.001$). In a multivariable model, although pretreatment $CD4^+$ T-cell count and plasma HIV RNA level were no longer predictive ($P > 0.17$ for both), higher pretreatment $CD4^+$ T-cell activation continued to independently predict higher month 12 $CD4^+$ T-cell activation ($P = 0.001$).

Predictors of mortality among HIV-infected Ugandans initiating antiretroviral therapy

A total of 34 Ugandan participants died after initiating ART, with an overall mortality rate of 4% (95% CI: 2–6%) at 6 months, 5% (95% CI: 3–7%) at 12 months, and 7% (95% CI: 5–10%) at 24 months. Because most of these deaths occurred without autopsies or other antecedent diagnostic testing, the causes of death are unknown. Each 100 cell/μl increase in pretherapy $CD4^+$ T-cell count was associated with a 51% (95% CI: 21–69%) decreased hazard of death ($P = 0.003$), largely driven by a high rate of death in the first 6 months of ART (Fig. 3a). There was no evidence for a relationship between pretherapy $CD4^+$ or $CD8^+$ T-cell activation, plasma HIV RNA level, sex, or age and overall mortality during ART ($P > 0.12$ for all). Fifteen participants died after month 6 of ART, 10 of whom had a confirmed plasma HIV RNA level less than 400 copies/ml at month 6 of therapy. These deaths occurred at a median of 18 months after treatment initiation (IQR: 15–21 months). Among participants with a plasma HIV RNA level less than 400 copies/ml at month 6 of ART, each 10% increase in month 6 $CD8^+$ T-cell activation was associated with a 1.65-fold increased hazard of subsequent death ($P = 0.029$, Supplementary Table 2, http://links.lww.com/QAD/A181 and Fig. 3b). Pretherapy $CD4^+$ T-cell count was less significantly associated with mortality after month 6 of ART (hazard ratio: 0.43, $P = 0.081$) and there was no evidence for a relationship between month 6 $CD4^+$ T-cell count and subsequent mortality (Fig. 3c) among those with plasma HIV RNA levels less than 400 copies/ml. Each 10% increase in month 6 $CD8^+$ T-cell activation continued to be associated with a 1.6-fold increased hazard of death even after adjustment for pretreatment $CD4^+$ T-cell count ($P = 0.048$) or month 6 CD4 count ($P = 0.042$).

Discussion

Immune activation is associated with poor ART-mediated $CD4^+$ T-cell recovery among HIV-infected individuals in resource-rich settings, but little is known about its prognostic importance in resource-limited settings, in which differences in host genetics, viral factors, and prevalent coinfections may modify these associations. Furthermore, no study has ever established a link between

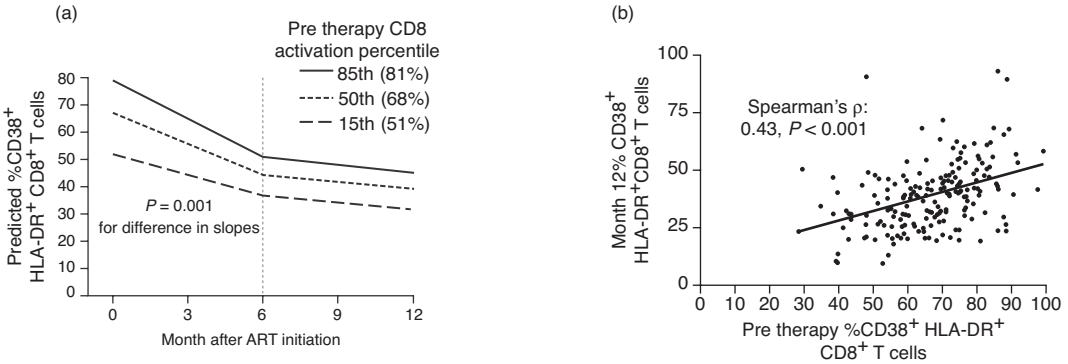

Fig. 2. Changes in $CD8^+$ T-cell activation during antiretroviral therapy-mediated viral suppression in HIV-infected Ugandans. (a) Mean predicted changes in the frequency of activated ($CD38^+$ $HLA\text{-}DR^+$) $CD8^+$ T cells was assessed over time with a linear mixed model among HIV-infected Ugandans achieving a plasma HIV RNA level less than 400 copies/ml by month 6 of antiretroviral therapy (ART). Predicted changes in T-cell activation are displayed when setting pretherapy $CD8^+$ T-cell activation to 15th (51%), 50th (68%), and 85th (81%) percentile values, *P* value representing whether the rate of decline varies according to pre-treatment T-cell activation level. (b) The relationship between pre-treatment and month 12 $CD8^+$ T-cell activation is plotted for participants maintaining a plasma HIV RNA level <400 copies/ml at month 12. The line represents predicted values from a linear regression model. *P* values are given for the non-parametric Spearman's rho statistic.

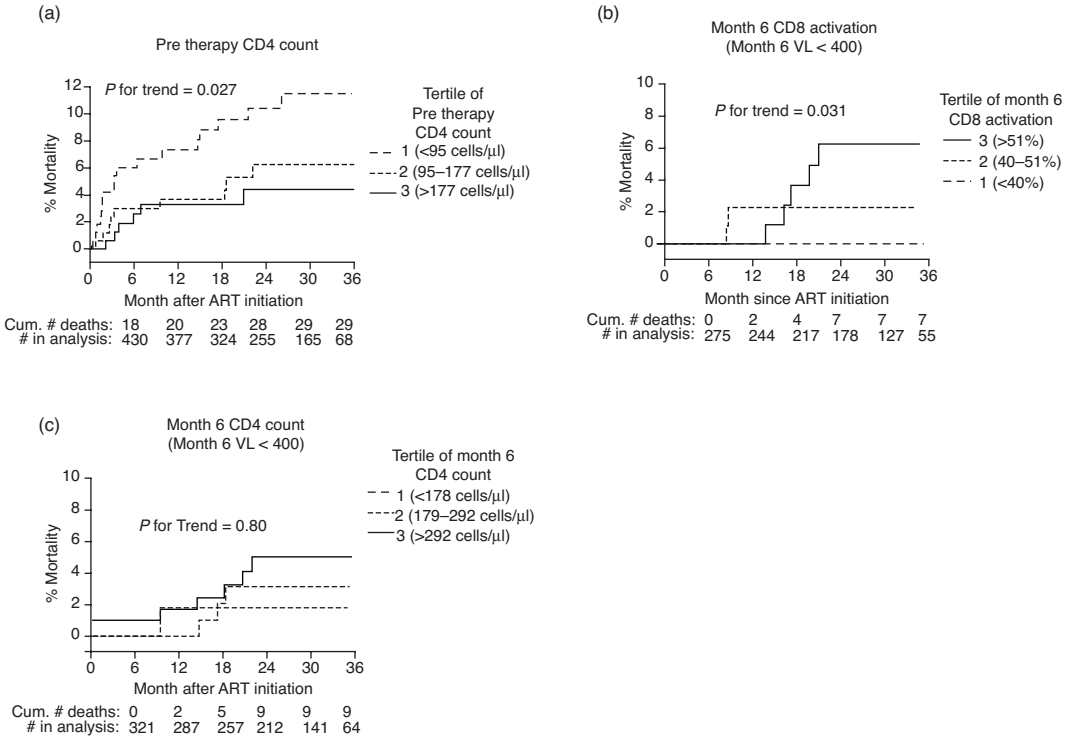

Fig. 3. Predictors of mortality during antiretroviral therapy among HIV-infected Ugandans. (a) Predictors of mortality were assessed using Kaplan–Meier methods among all HIV-infected Ugandans initiating antiretroviral therapy (ART) as well as the subset achieving a plasma HIV RNA level less than 400 copies/ml by month 6 of ART. Predictors were assessed in tertiles. Lower pretherapy $CD4^+$ T-cell counts predicted earlier death among all participants, largely driven by deaths occurring in the first 6 months of ART (P for trend = 0.027. (b) Among participants with plasma HIV RNA levels less than 400 copies/ml at month 6 of ART, higher $CD8^+$ T-cell activation predicted earlier subsequent mortality (P for trend = 0.031). (c) However, there was no evidence for a relationship between month 6 $CD4^+$ T-cell count and subsequent mortality among participants with plasma HIV RNA levels less than 400 copies/ml (P for trend = 0.80).

persistent T-cell activation during suppressive ART and clinical morbidity or mortality. In the current study, we have established that higher pretreatment $CD8^+$ T-cell activation (and a trend for month 12 T-cell activation) independently predicts slower subsequent $CD4^+$ T-cell recovery. Furthermore, we have established for the first time that higher persistent $CD8^+$ T-cell activation during early viral suppression independently predicts subsequent mortality in this setting, suggesting that immune activation is a major determinant of clinical outcomes in resource-limited settings and providing important clinical validation for these markers in the setting of viral suppression.

It has long been appreciated that HIV-uninfected individuals living in resource-limited settings in sub-Saharan Africa have higher frequencies of activated T cells than those living in resource-rich settings [22–27]. These differences have often been attributed to environmental factors (including prevalent helminthes infections [22,25]), as Africans living in resource-rich countries for many years often have lower T-cell activation levels than those living in Africa [24,25], and Europeans living in sub-Saharan Africa often exhibit higher T-cell activation levels than those living in Europe [24]. The mechanisms explaining the higher $CD8^+$ T-cell activation levels in sub-Saharan Africans remains unclear, but may include prevalent coinfections (i.e., helminthes, subclinical malaria, etc.), or differences in host genetics or viral clade (although our group has not observed differences in T-cell activation among HIV-infected Ugandans infected with clades A and D [28]).

Our finding that higher pretherapy $CD8^+$ T-cell activation predicts slower $CD4^+$ T-cell recovery in HIV-infected Ugandans is consistent with several prior studies in North America and Africa linking T-cell activation to poor $CD4^+$ T-cell recovery [7,8,10,29]. However, only one of these prior studies established a temporal relationship between T-cell activation and subsequent $CD4^+$ T-cell recovery [10]; thus, our current study adds important evidence supporting a causal role of immune activation in $CD4^+$ T-cell recovery. These data further suggest that the immune activation levels

established prior to the initiation of therapy have a durable impact on the capacity for $CD4^+$ T-cell recovery long after viral suppression is achieved. The mechanisms explaining the durable impact of pretreatment immune activation on $CD4^+$ T-cell recovery remain unclear, but could involve immune activation-induced lymphoid fibrosis, which fails to normalize during suppressive ART and has been shown to impair naive and central memory $CD4^+$ T-cell homeostasis and $CD4^+$ T-cell recovery in this setting [30]. Alternatively, immune activation levels prior to ART may simply predict the extent of persistent immune activation during suppressive therapy. Our data provide less support for this model because the relationship between month 12 $CD8^+$ T-cell activation and subsequent $CD4^+$ T-cell recovery was not formally significant ($P = 0.075$) and there was no evidence for a relationship between month 6 $CD8^+$ T-cell activation and subsequent $CD4^+$ T-cell recovery, although the much smaller number of participants contributing to these analyses resulted in comparatively lower statistical power and the β coefficient for the pre-ART and month 12 $CD8^+$ T-cell activation-time interaction terms were identical (-0.2). Longitudinal data from larger cohorts of HIV-infected individuals maintaining treatment-mediated viral suppression will be necessary to resolve this issue. Lastly, it is possible that prevalent opportunistic infections at the time of ART initiation may have contributed to both higher levels of $CD8^+$ T-cell activation and poor $CD4^+$ T-cell recovery. However, given limited diagnostic testing available in the resource-constrained clinic from which the participants were sampled, we do not have comprehensive laboratory-confirmed opportunistic infection data available for this cohort. However, as pretreatment $CD8^+$ T-cell activation primarily affected late $CD4^+$ T-cell recovery (after the first year of ART), it is unlikely that many opportunistic infections present at ART initiation would have remained untreated for over a year, affecting late $CD4^+$ T-cell recovery.

We also found that higher pretreatment T-cell activation and lower pretreatment $CD4^+$ T-cell counts strongly predicted higher T-cell activation during suppressive ART. This is an important observation as it may suggest an immunologic cost to delaying ART initiation in this setting. The fact that pretreatment T-cell activation strongly predicts T-cell activation levels during suppressive ART also suggests that factors other than the extent of productive HIV replication are likely to contribute to T-cell activation both in the presence and absence of suppressive ART. Studies in both resource-rich and resource-limited settings suggest that residual T-cell activation during ART-mediated viral suppression may be at least partly explained by persistently abnormal levels of microbial translocation [31–33] and other chronic coinfections including cytomegalovirus and/or other herpesviruses [16]. It is conceivable that the extent of immunodeficiency prior to ART initiation may be a major determinant of both microbial translocation and herpesvirus replication once ART-mediated viral suppression is achieved, but this has yet to be established in longitudinal studies. Prevalent coinfections in East Africa (i.e., helminthes, subclinical malaria, and so on) are also likely to contribute to immune activation in both the presence and absence of ART. Host genetic factors may also modulate the inflammatory response to both productive HIV replication in the absence of ART and release of HIV from latently infected cells during suppressive ART. However, the host genetic determinants of immune activation have yet to be established in HIV infection.

Lastly, we observed for the first time that higher $CD8^+$ T-cell activation levels during early ART-mediated viral suppression independently predicts subsequent mortality. Although low pretherapy $CD4^+$ T-cell counts remains the strongest predictor of overall mortality in HIV-infected Ugandans, high persistent $CD8^+$ T-cell activation during suppressive ART is a more significant predictor of deaths occurring after the first 6 months of therapy. This association is unlikely to be mediated by incomplete viral suppression because this analysis was restricted to those with undetectable plasma HIV RNA levels at month 6 of ART. Furthermore, high persistent $CD8^+$ T-cell activation levels at month 6 are unlikely to be the immediate consequence of an undiagnosed fatal infection because the majority of deaths occurred at least 12 months after the T-cell activation measurement. However, this observation is based on a relatively small number of deaths and we did not observe any evidence for a relationship between pre-ART $CD8^+$ T-cell activation and mortality (although the extent of pre-ART $CD8^+$ T-cell activation may have been in part driven by the degree of viremia, which was not associated with subsequent mortality in our study). Although the relationship between month 6 $CD8^+$ T-cell activation and subsequent mortality will need to be confirmed in larger and/or longer studies, it suggests clinical relevance to the abnormally high T-cell activation observed in treated HIV-infected Ugandans and suggests that additional interventions designed to decrease residual T-cell activation may be required to fully restore health in this setting.

In summary, high pre-ART $CD8^+$ T-cell activation independently predicts a diminished rate of late $CD4^+$ T-cell recovery in HIV-infected Ugandans maintaining treatment-mediated viral suppression. Higher persistent $CD8^+$ T-cell activation during early treatment-mediated viral suppression also independently predicts earlier mortality. Thus, interventions designed to further reduce immune activation in this setting should be studied. Given the strong relationship between lower pretreatment $CD4^+$ T-cell count and higher residual T-cell activation, these observations also support the earlier initiation of ART in this setting.

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Conflicts of interest

There is no conflict of interest for any of the investigations involved in this study.

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