Predictors of virologic response in persons who start antiretroviral therapy during recent HIV infection. Maile Y. Karris, Yu-ting Kao, Derek Patel, Matthew Dawson, Steven P. Woods, Florin Vaida, Celsa Spina, Douglas Richman, Susan Little and Davey M. Smith University of California San Diego, La Jolla, and Veterans Administration San Diego Healthcare System, San Diego, California, USA. AIDS. 2014 Jan 3. [Epub ahead of print]

Introduction

Starting ART within the first year of HIV infection decrease transmission, lower the immune activation setpoint, minimize latent cellular reservoirs, improve B- cell function, and normalize CD4 cell counts. A growing consensus supports this approach, despite open questions about the 'personal health benefit'.

To be maximally effective, ART should suppress viral replication to as low as possible as fast as possible. HIV replication in the presence of ART delays immune reconstitution and increases the risk of drug resistance. This can be especially important when ART is started during the earliest stages of HIV infection, which is marked by very high viral loads and levels of immune activation.

Objective

To evaluate sociodemographic, immunologic, and virologic factors associated with suppression of HIV RNA levels on ART (<40 copies/ml) during recent infection.

Methods

Study design

Observational study nested within the San Diego Primary Infection Cohort (SD PIC).

Study participants

Participants were identified through two HIV screening campaigns. All participants underwent informed consent as approved by the local IRB. An estimated date of infection (EDI) was determined for all participants, according to the SD PIC algorithm (detuned HIV enzyme immunoassays, HIV Western blot, and HIV-1 nucleic acid testing).

Study procedures

ART was generally encouraged and participants could choose to start ART at any time after study entry. HIV drug resistance tests were performed at baseline visit.

The following procedures were performed at weeks 0 and 24:

- Assessments of alcohol (Alcohol Use Disorders Identification Test, AUDIT) and methamphetamine use (Drug Abuse Screening Test, DAST),

- Clinical laboratory: CD4 and CD8 T-cell subsets and HIV RNA levels
- Immunologic investigation – peripheral blood mononuclear cells (PBMC): Fresh peripheral blood was processed to obtain viable PBMCs; PBMCs were aliquoted with staining buffer prior to incubation with conjugated antibodies to CD3, CD4, CD8, CD45RO, CD27, CCR5, CCR7, HLA-DR, CD38, CCR6, and CXCR5. Samples were run on a BD FACSCanto II instrument and data analyzed with FlowJo software.
- Immunological investigation – gut cells: participants who consented to this procedure underwent standard colonoscopy with 10 – 20 punch biopsies obtained at the ileal-cecal and rectosigmoid junctions at week 0, 12, 24, and 48. The biopsies were immediately processed to produce single cell suspensions prior to being frozen. The samples were evaluated by flow cytometry, after all samples had been collected. Conjugated antibodies added to the single cell suspensions included CD3, CD4, CD8, CD45RO, CD27, CCR5, CCR7), IgG_1, and Ki67. Samples were run and data analyzed using the same instrument and software used for PBMC.
- Markers of microbial translocation: measurement of lipopolysaccharide and soluble CD14 (sCD14).

Statistical analyses

. For analyses involving participants who achieved or not undetectable viral load at week 24 the analyses was repeated by removing all study participants who had less than 4 weeks of ART by study end.
. Linear regression was used for the association between lymphocyte factors and continuous predictors (age, sCD14). Logistic regression was used for the association between virologic response at week 24 and lymphocyte factors.

Results

From October 2009 to April 2011, 46 men were enrolled into the study (figure 1), from whom 29 (69%) chose to start ART at any time during the study period. Median estimated time of infection was 6.2 weeks. The participants had a median age of 28 years, 84% identified themselves as white, 35% reported Hispanic ethnicity. The median viral load was 4.95 $\log_{10}$ HIV RNA copies/ml and CD4 cell count was 609 cells/ml. Demographic characteristics, alcohol and methamphetamine use did not differ in persons who started ART and those who did not.

Characteristics of persons who achieved a virologic response

Of the 29 study participants who started ART, 17 (58.6%) achieved Viral Load <40 copies/ml in 24-week (Table 1). Three (10%) initiated a NNRTI, six (21%) integrase inhibitor, and 20 (69%) a boosted PI, all combined with tenofovir and emtricitabine. ART regimen and baseline drug resistance was not predictive of achieving undetectable VL

by study end. Persons who achieved undetectable VL were more likely to have started ART earlier in their course of HIV infection (median 11.9 weeks from EDI vs. 20 weeks, P = 0.03) and were younger (median 27 vs. 35 years, P = 0.07). There were no statistical differences in other sociodemographic factors, alcohol or methamphetamine abuse, baseline HIV RNA levels and CD4 cell counts. All persons on ART were 100% adherent by self-report. Multivariable logistic regression showed that lower sCD14 levels and younger age were independently predictive of achieving undetectable HIV RNA (Fig. 2a and b: boxplotting showing baseline median sCD14 and age in the 2 groups, Table 2: shows Odds Ratio for the variables analysed).

Gut-associated lymphoid tissue

Seven participants had gut biopsies available, and all chose to start immediate ART. Their median estimated time of infection was 3.4 weeks, and the median time on ART was 23 weeks. Only one participant did not achieve an undetectable VL at 24 weeks. Numbers of GALT CD4 T cells did not increase over time (Fig. 3a). Figures 3b-d show a significant decrease of CD4 and CD8 central memory T cells and an increase of CD8 effector T cells. No significant associations were observed between duration of infection prior to ART and modulation of T-cell phenotypic subsets or proliferation at week 24.

Soluble CD14 as a predictor of virologic response

No correlation was identified between sCD14 and GALT immunologic factors at baseline. At week 24, sCD14 levels were negatively correlated with proportions of central and effector memory CD4 T cells, and effector memory CD8 T cells in the blood.

Age as a predictor of virologic response

Baseline naive CD4 T cells was negatively correlated with age in the multivariable analysis. There was no correlation between age and cellular immune activation, regulatory T cells, immunosenescent T cells, LPS, or sCD14 at any time point.

Immunologic factors associated with virologic response

Participants who achieved undetectable VL had, at baseline: 1)lower proportions of central memory CD4 T cells and of proliferating central memory CD4 T cells; 2) higher percentages of central memory, and effector memory CD8 T cells, as well as higher proportions of activated effector memory, and activated and proliferating effector CD8 T cells. This could suggest that a more robust effector CD8 T-cell response contributes to a virologic response, but after adjusting for baseline covariates (age, EDI to ART, weeks on ART, $\log_{10}$ HIV viral load, CD4 cell, sCD14, and LPS) in logistic regression, there were no immunologic factors that remained significantly different between groups.

Discussion

Both lower levels of sCD14 and younger age at initiation of ART during recent infection were predictive of who achieved undetectable plasma HIV RNA, but the exact mechanisms underlying the negative association of age and microbial translocation with the ability to achieve rapid viral suppression remain unclear.

HIV enteropathy is associated with increased intestinal permeability and microbial translocation; sCD14 and LPS (markers of microbial translocation) have been described as independently predictive of HIV mortality and disease progression and associate to viral load. This is the first study to link baseline sCD14 with virologic response during ART.

Starting ART in early HIV infection did not improve recovery of CD4 T-cell proportions in GALT, despite all participants starting ART within 30 days of EDI. Thus, the '4-week window of opportunity' to start ART to achieve normal CD4 T-cell recovery does not appear to apply to this cell population, this may in part be due to collagen deposition preventing repopulation of the GALT. The significant and continued loss of central memory CD4 T cells over time in the GALT, could possibly be due to local ongoing HIV replication in GALT, or due to cell-mediated death of latently infected CD4 T cells, especially in the presence of significantly increasing proportions of effector CD8 T cells.

Previous studies have associated younger age with virologic failure, attributed to better adherence and engagement in care in older persons. Studies of the effect of age on the progression of HIV infection do suggest older persons have a poorer prognosis and higher risk of immunologic failure, probably due thymic deterioration and a reduced capacity to generate new CD4 T cells. This is supported by this study, in which age was negatively correlated with percentages of naive CD4 and CD8 T cells in peripheral circulation and positively correlated with more mature CD4 and CD8 T-cell subsets (central memory and effector memory). Control of HIV replication is dependent on the availability of naive T cells, degree of T- cell avidity, functionality, and clonal turnover, qualities that are also affected by age. Specific evaluation of T-cell functionality was not assessed, so a clear pathogenic reason for the observed association between older age and prolonged viremia could not be discussed.

Future studies, specifically in persons with acute HIV infection, may lead to a further understanding of whether there truly is an immunologic benefit of using more potent ART regimens and GALT-protective regimens in this population.

Limitations

1) Not a RCT, Individual differences in time to ART; 2) Small sample size limits the power to detect GALT factors associated with viral suppression; 3) Drug levels, kidney and liver functions not evaluated; 4) HIV RNA and DNA, HIV-specific CD8 T cells in GALT not measured