HIV Antibodies Decline During Antiretroviral Therapy but Remain Correlated With HIV DNA and HIV-Specific T-Cell Responses

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Background: In people with HIV on antiretroviral therapy (ART), the relationship between HIV-specific immune responses and measures of HIV persistence is uncertain.

Methods: We evaluated 101 individuals on suppressive ART in the AIDS Clinical Trials Group A5321 cohort. Cell-associated (CA) HIV DNA and RNA levels and HIV antibody concentrations and avidity to Env/p24 were measured longitudinally at years 1, 4, and 6–15 after ART initiation. Plasma HIV RNA by single copy assay and T-cell responses (IFN-γ ELISPOT) against multiple HIV antigens were measured at the last time point.

Results: HIV antibody levels declined significantly with increasing time on ART (19%/year between year 1 and 4). HIV antibody levels correlated with T-cell responses to HIV Pol (r = 0.28, *P* = 0.014) and to Nef/Tat/Rev (r = 0.34; *P* = 0.002). HIV antibody and T-cell responses were positively associated with HIV DNA levels; for example, at the last time point (median 7 years on ART), r = 0.35 for antibody levels and HIV DNA (*P* < 0.001); r = 0.23 for Nef/Tat/Rev-specific T-cell responses and HIV DNA (*P* = 0.03). Neither antibody nor T-cell responses correlated with cell-associated HIV RNA or plasma RNA by single copy assay.

Conclusions: In individuals on long-term ART, HIV-specific antibody and T-cell responses correlate with each other and with HIV DNA levels. The positive correlation between HIV immune responses and HIV DNA implies that the immune system is sensing, but not clearing, infected cells, perhaps because of immune dysfunction. Measuring immune responses to HIV antigens may provide insight into the impact of reservoir-reducing strategies.

Key Words: HIV antibodies, HIV persistence, HIV T-cell responses, HIV reservoir

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INTRODUCTION

In people with HIV, antiretroviral therapy (ART) suppresses plasma HIV RNA to levels below the limits of detection of commercial assays,[1] but a reservoir of latently infected cells persists that leads to HIV rebound if ART is stopped.[2] A measure of the latently infected cell population in people on ART is cell-associated HIV DNA, which is comprised of intact and defective proviruses. Because defective proviruses rapidly accumulate soon after HIV acquisition, only a small fraction of proviruses is intact[3] and potentially replication-competent; as a result, HIV DNA is considered to be a maximal measure of the HIV reservoir. Latently infected HIV DNA-containing cells have previously been thought to not express antigen and, therefore, to be invisible to the immune system. There are recent data, however, indicating that intact and defective HIV proviruses

are transcribed, leading to the possibility of intermittent antigen expression in people on ART.[4]

HIV-specific immune responses usually decline after ART is initiated, because of decreasing levels of viral antigen.[5,6] For example, over the first one to 2 years of ART, HIV-specific CD8 T-cell responses declined by median 52% per year (half-life 49.8 weeks, based on 5 individuals).[7]

However, if there is intermittent antigen expression and recognition, HIV antibody and T-cell responses may be elicited and maintained. Understanding the longitudinal relationship between HIV-specific immune responses and measures of HIV persistence may provide insight into whether the immune response continues to sense infected cells in people on ART. In addition, if HIV-specific immune responses correlate with the number of infected cells during ART, tracking these responses may provide information on the impact of interventions designed to stimulate virus expression or reduce HIV reservoirs.[8–10] To understand the relationship between HIV-specific antibody responses and measures of HIV persistence, we conducted a longitudinal study in a large cohort of participants on long-term suppressive ART.

METHODS

Study Population

We evaluated participants with chronic HIV infection who initiated ART in AIDS Clinical Trials Group (ACTG) studies and had subsequent follow-up specimens while continuing to receive ART (ACTG studies A5001 and A5321[11]). Participants had HIV RNA <50 copies/mL by commercial assays at or before week 48 of ART and at all subsequent time points and no reported ART interruptions. Informed consent was obtained from participants, and human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of clinical research.

Virologic Assays

Cell-associated (CA) HIV DNA and unspliced HIV RNA levels (using quantitative PCR targeting HIV pol) in peripheral blood mononuclear cells were measured longitudinally before initiation of ART and on-ART years 1, 4, and (for some participants) 6–15 years using previously described methods.[11,12] Primers and probes used for qPCR of HIV DNA, CA-RNA, and plasma HIV RNA were identical. Testing of the cellular housekeeping gene, IPO8, served as an internal control for mRNA recovery. Results were expressed as copies/million $CD4^+$ T-cells normalized for % $CD4^+$T-cells in blood. Plasma HIV RNA by single copy assay (SCA) was measured on samples from the last on-ART time point using a previously reported method.[13]

HIV Antibody Assays

HIV antibodies were measured at years 1, 4 and, for some participants, years 6–15 after ART initiation using assays that have been previously described.[8] Less-sensitive (LS) and Avidity-modified VITROS HIV 1 + 2 were used to measure antibodies against HIV envelope (Env) and p24 (Gag).[8] Limiting Antigen (Lag)-Avidity EIA was performed as previously described.[14] The assays for HIV antibodies used in this study are FDA-cleared diagnostic tests that provide reproducible clinical measurements over time and across assay lots; the performance characteristics have been published.[15,16]

T-Cell Responses Measured by IFN-γ ELISPOT

HIV interferon-gamma ELISPOT assays were performed as previously described and reported on samples from the last on-ART time point.[9] In brief, Multiscreen IP 96-well plates (Millipore) were coated with 0.5 µg/mL of anti-interferon-gamma antibody (clone 1-D1K, MAbtech, Sweden) in phosphate-buffered saline and incubated overnight. Plates were washed, peripheral blood mononuclear cells were added at 2×10^5 cells per well and HIV peptide pools (10 µg/mL/peptide) and phytohemaggultinin (2 µg/mL) were added. Plates were incubated overnight, washed and secondary antibody was added (clone 7-B6-1, Mabtech) and incubated for 1 hour. Plates were developed with Streptavidin-ALP (MAbtech) and developed with Color Development buffer (Bio-Rad, Hercules, CA). Plates were washed, dried overnight and spots were counted.

Statistical Analysis

Rank-based correlations (Spearman) and rank-sum test were used. Results below assay limits were assigned the lowest rank. Within-participant change per year between years 1–4 on ART in log10-transformed measures of HIV antibody were estimated with participant-specific linear regression models. Changes over time were compared against the null hypothesis of no change using the Wilcoxon signed-rank test.

RESULTS

Study Population

A total of 101 participants who initiated ART underwent longitudinal testing of HIV antibodies and measurements of HIV DNA and CA-RNA. The median age at ART initiation was 39 years; 21% were female. Participants had a median of 7 years on ART (minimum, maximum: 4, 15) at the time of the last sample collection. Median pre-ART $CD4^+$ and $CD8^+$ T-cell counts were 290 and 792 cells/mm³, respectively. At the time of the last blood collection, median $CD4^+$ and $CD8^+$ T-cell counts were 681 and 699 cells/mm³, respectively. Median pre-ART HIV RNA was 4.6 log_{10} copies/mL. All participants had plasma HIV RNA levels <50 copies/mL at all time points at or after week 48 of ART. Additional details regarding the cohort have been previously published.[11]

HIV Antibodies Decline During Long-Term ART

There was a strong correlation between antibody level (as measured by signal to cutoff ratio) and avidity at each on-treatment time point (all r ≥ 0.95, P < 0.001; see

Supplemental Figure 1, Supplemental Digital Content, http://links.lww.com/QAI/B327).

HIV antibodies declined significantly with increasing time on ART (Fig. 1A): between years 1 and 4 on ART, antibody levels declined by median 19%/year and avidity declined by median 5.6%/year (*P*-values < 0.001). Ninety-nine percent of participants had a negative antibody level slope and 96% had a negative avidity slope. Participants with higher HIV antibody levels and avidity at year 1 of ART had higher levels and avidity at year 4 of ART (r = 0.97 and 0.96, respectively; *P* < 0.001) (see Supplemental Figure 2, Supplemental Digital Content 2, http://links.lww.com/QAI/B327).

HIV Antibodies and Pre-ART Characteristics

At year 1 of ART, higher HIV antibody levels and avidity were modestly correlated with older age (r = 0.23 and r = 0.23, *P* < 0.05, respectively), but not participant sex (*P* ≥ 0.37). Of the 101 participants, 21% were female; this number may be too low to assess differences in antibody responses by sex. There were no significant correlations observed between HIV antibody level or avidity at year 1 on ART with pre-ART plasma HIV RNA, pre-ART HIV DNA, pre-ART CA-RNA, pre-ART CD4 cell count, or pre-ART CD4:CD8 ratio (all *P*-values ≥ 0.08).

HIV Antibody Responses Correlate With T-Cell Responses Directed Against Nef/Tat/Rev and Pol

HIV ELISPOT assays were performed at the last time point after ART initiation (median of 7 years) and compared with antibody levels and avidity at the same time point (Fig. 2). HIV antibody and avidity correlated with T-cell responses to all HIV peptides (r = 0.27, *P* = 0.02 and r = 0.27, *P* = 0.02, respectively). HIV antibody levels and avidity also correlated with T-cell responses to HIV Pol (r = 0.28, *P* = 0.014 and r = 0.25, *P* = 0.024, respectively) and to Nef/Tat/Rev (r = 0.34, *P* = 0.002 and r = 0.34, *P* = 0.002). There were no correlations between HIV antibody and T-cell responses to HIV Gag or Env, or to CMV and EBV controls.

HIV Antibody and T-Cell Responses on ART Correlate With HIV DNA

At year 1 of ART, HIV antibody level and avidity were positively correlated with HIV DNA (r = 0.24 and 0.27, respectively; *P* < 0.02). Similarly, at year 4 on ART, both HIV antibody measures were correlated with HIV DNA (r = 0.31 and 0.36, *P* ≤ 0.002, respectively). At the last time point (median of 7 years on ART), antibody level and avidity
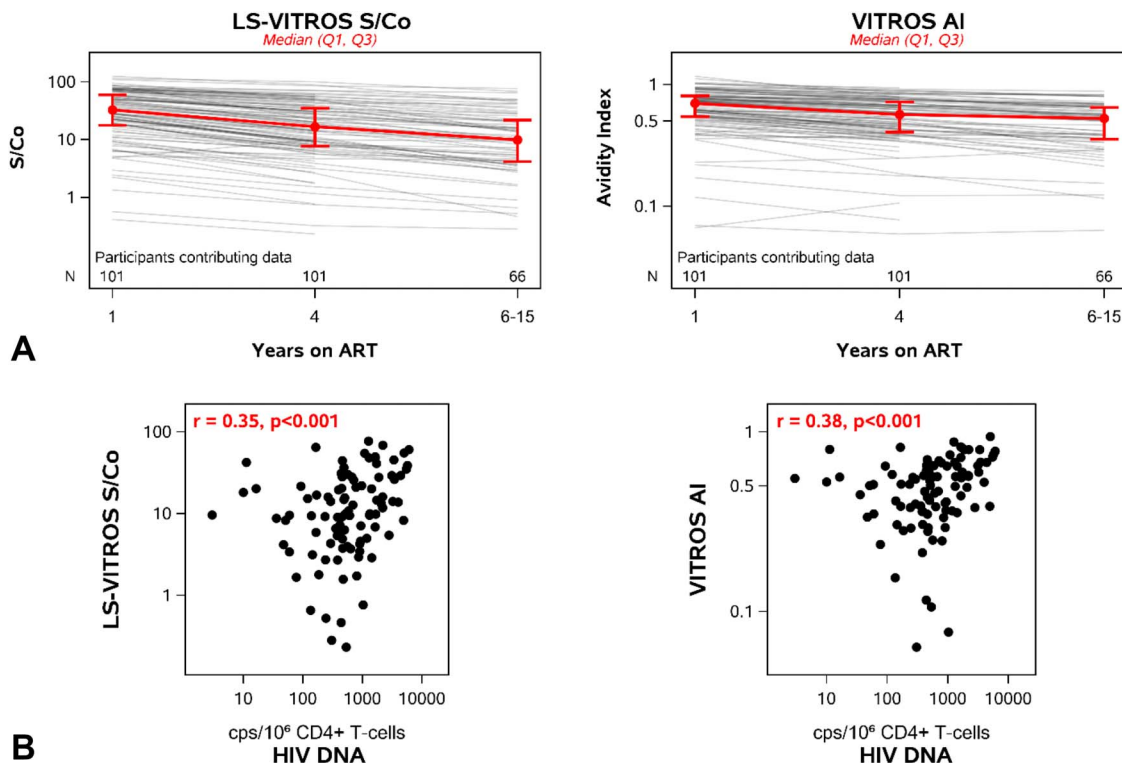
FIGURE 1. HIV antibody levels and avidity decline with time on antiretroviral treatment and correlate with HIV DNA levels. Lines correspond to longitudinal antibody responses for each participant (n = 101). Gray lines represent the median and interquartile range. Antibody levels were measured using the LS-Vitros signal/cut-off (S/Co) ratio. Antibody avidity was measured by the vitros avidity index (AI). A, LS-VITROS S/co and AI results over time on ART. Between years 1 and 4 on ART, antibody levels declined by median 19%/year (1.23-fold/year) and avidity declined by median 5.6%/year (1.06-fold/year) (*P*-values < 0.001). B, Correlation of LS-VITROS and AI with HIV DNA/10^6 CD4$^+$T cells at the last longitudinal time point (n = 96).

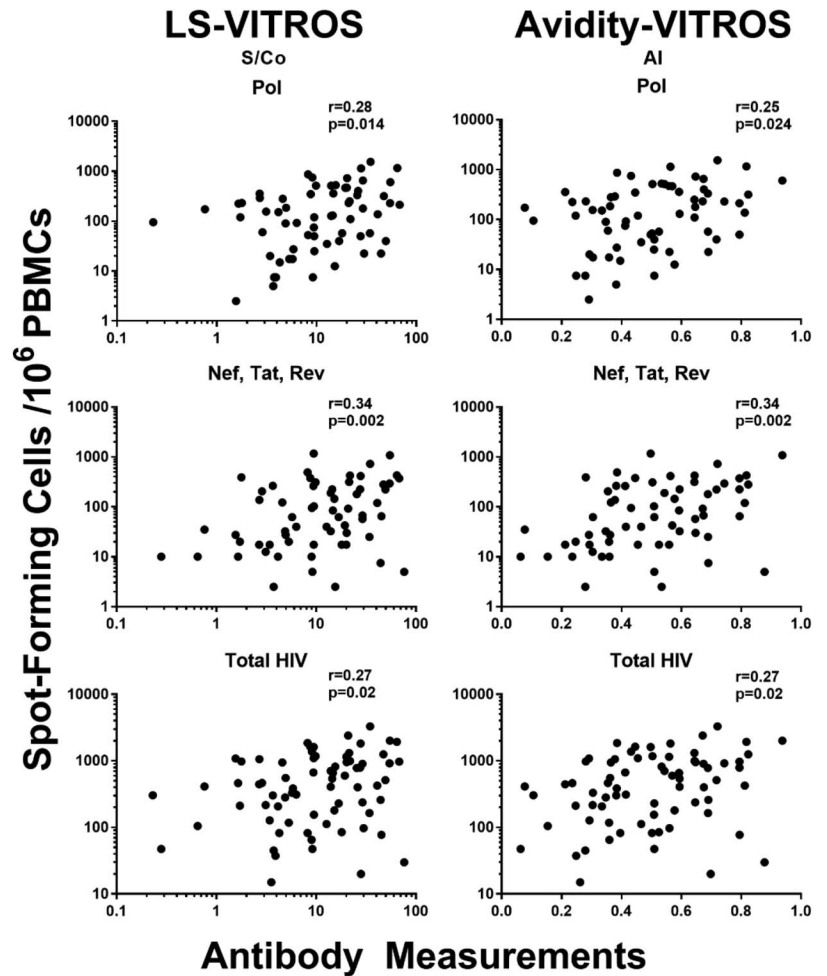**FIGURE 2.** HIV antibody levels and avidity correlate with HIV antigen-specific T-cell responses. Each data point represents the antibody measurements or the number of antigen-specific IFN-γ spot-forming T-cell responses for each participant. Shown are correlations between IFN-γ-producing HIV-specific T-cell responses and LS-VITROS or AI measurements of antibody. Top panels are T-cell responses to pol, middle panels are responses to Nef/Tat/Rev, and bottom panels are T-cell responses to total HIV.

continued to be correlated with HIV DNA levels (r = 0.35 and 0.38, respectively, $P < 0.001$; Fig. 1B). Similarly, at the last time point, Nef/Tat/Rev-specific T-cell responses correlated with HIV DNA levels (r = 0.23, $P = 0.03$), as previously reported.[9]

HIV antibody levels and avidity were not significantly correlated with CA-RNA (measured at years 1 and 4; $P \geq 0.24$) and were not significantly correlated with plasma HIV RNA by SCA (measured at year 4–15). Similar to the results for HIV antibodies, at the last time point (as previously reported[9]), T-cell responses did not correlate with CA-RNA or plasma HIV RNA by SCA.

DISCUSSION

In this longitudinal study, we found that HIV antibody levels and avidity declined significantly during ART. HIV antibody levels correlated with T-cell responses to HIV Pol and Nef/Tat/Rev. Despite many years of ART, during which plasma HIV RNA was consistently suppressed to <50 copies/mL, we found that HIV antibody and T-cell responses were positively correlated with HIV DNA levels. The positive correlation between HIV immune responses and HIV DNA implies that the immune system is sensing, but not clearing, infected cells, perhaps because of functional defects in immune responses or resistance of infected cells to elimination (both of which have been described in previous studies in ART-treated individuals.[17,18])

Before initiation of ART, viral replication and antigen production induce HIV-specific antibody and T-cell responses. The decline in immune responses after initiation of ART is expected, given the reduced levels of HIV production in persons on suppressive ART. In fact, the latently infected cell population that persists in people on long-term ART has been thought to be invisible to the immune system. Some HIV-infected cells, however, are releasing intact viral particles that can be detected in plasma by single-copy assay,[1] and recent findings that defective HIV proviruses are transcribed provides another avenue by which antigen expression may occur in people on ART.[4] Our findings that HIV antibody responses and our previous report that T-cell responses[9] correlate with HIV DNA levels supports the hypothesis that there is intermittent antigen production during ART[19] that maintains immune responses against the virus.

A strength of this study is that participants had documented sustained suppression of plasma HIV RNA for

many years after treatment initiation, allowing us to assess the relationship between immune responses and HIV persistence without potential confounding that may result from transient detectable viremia or virologic failure. The finding that antibody levels and avidity—and the previous finding that HIV-specific T-cell responses—are correlated with HIV DNA, but not CA-RNA or low-level plasma viremia, was unexpected. One potential explanation is that the number of infected cells, as measured by HIV DNA, reflects the likelihood of intermittent antigen production and induction of immune responses better than CA-RNA, because of blocks in nuclear export or translation. Another possibility is that blood levels of CA-RNA and HIV RNA by SCA may not reflect cumulative in vivo antigenicity, perhaps because of fluctuations in RNA levels over time, varying copy numbers per cell and/or antigen production in lymphoid or other compartments.

The current findings should be considered in light of the results of a previous study performed on the same set of samples, which reported a direct correlation between HIV-specific T-cell responses and HIV DNA.[9] The correlation was unique to T-cell responses directed against Nef, with no correlations detected for T-cell responses to other gene products, including Gag and Env. In this study, there was a correlation between antibody responses to Gag/Env and HIV DNA (we did not measure antibody responses to Nef). Gag/Env-specific antibody responses correlated strongly with Nef-specific T-cell responses, but not with Gag- or Env-specific T-cell responses. We propose 2 potential explanations: (1) Nef-mediated immune-evasion through MHC class I down-regulation[20] may prevent T cells, but not B cells, from sensing late gene products, such as Gag and Env, produced by infected HIV DNA-positive cells; (2) long-term antigen exposure and paucity of CD8 cells to clear HIV-infected cells in the B cell follicle may drive a differential B and T-cell immune response.[21] Further study is needed to distinguish among these and other possibilities.

HIV antibodies have been proposed as a method to monitor HIV persistence in people on ART.[8] Here we show that there are significant declines in antibody levels and avidity in almost all persons on ART, likely because of the loss of stimulating antigens.[22] One limitation is that we were not able to test pre-ART specimens for antibody; as a result, we cannot assess changes in these measures during the first year of ART. However, a cross-sectional analysis of a separate cohort by our group supports the finding that antibodies correlate with measures of HIV persistence during treatment.[8] In addition, quantitative HIV-specific antibodies were recently reported to correlate with HIV DNA levels in children on ART,[10] leading the authors to similarly propose their use for monitoring HIV persistence.

Another study limitation is that the cohort started ART at a lower CD4 cell count (median 290/mm^3) than is typical currently and with regimens that include older medications. This CD4 cell count nadir may be lower than current values, but there is still a substantial proportion of people in the United States who have a CD4 cell count <200/mm^3 at time of diagnosis (about 25% in a recent study).[23] In ART regimen, approximately 50% of study participants received

a non-nucleoside reverse transcriptase inhibitor, 30% received a protease inhibitor, and 20% received an integrase inhibitor.[11] Even though integrase inhibitors are used more widely now than in the past, differences in regimen are unlikely to affect immune responses in people with sustained virologic suppression, as in our cohort.

In conclusion, in persons on long-term ART, HIV-specific antibody and T-cell responses correlate with each other and with HIV DNA levels. The positive correlation between HIV immune responses and HIV DNA suggests that the immune system may be periodically sensing, but not efficiently clearing, infected cells, perhaps because of immune dysfunction. Sensing of infected cells by immune responses suggests that tracking these measures may be a method of assessing the impact of novel reservoir-reducing strategies.

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