Early ART treatment prevents B cell loss and dysfunction in people living with HIV

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BACKGROUND

Peripheral B cell counts significantly decline starting 2 weeks after initial HIV-1 infection in the absence of ART. However, few studies have characterized longitudinal B cell phenotypic changes after early ART initiation. Here, we compare longitudinal changes in B cell activation and differentiation in PLWH in the presence and absence of ART starting from early acute infection in both the periphery and mucosal compartments.

METHODS

Specimens from participants in the RV217 Early Capture HIV Cohort (ECHO) and RV254 Acute HIV Infection (AHI) study were used to assess B cell changes in PLWH who are therapy-naïve or treated during early AHI, respectively. Flow cytometry was employed to determine B cell counts and phenotypes in both peripheral blood mononuclear cells (PBMC) and mucosal mononuclear cells (MMC) isolated from sigmoid biopsies at early and chronic infection time points including weeks 0 (AHI diagnosis), 2-4, 12, 24, and 52-96 after infection and in the presence or absence of ART. Healthy donors (RV304) or pre-infection time points (RV217) served as controls.

RESULTS

- PLWH have reduced peripheral B cell counts 2 weeks following HIV-1 RNA+ diagnosis; however, administration of ART during AHI resulted in higher peripheral B cell counts compared to untreated PLWH ($p<0.05$) (Fig. 1).
- Following ART, PLWH treated during Fiebig stage II or III showed significantly increased peripheral B cells (Fig 1, $p<0.01$), whereas PLWH treated during Fiebig stage I maintained similar B cell counts ($p>0.05$), indicating that peripheral B cell loss has not yet occurred in Fiebig 1 captured donors.
- In the absence of ART, the frequency of peripheral memory B cells decreased and plasmablast populations increased 2 weeks after infection ($p<0.01$) compared to uninfected time points (Fig. 2). However, early administration of ART prevented these phenotype B cell changes at all tested time points ($p>0.05$).
- Conversely, compared to healthy donors, frequencies of mucosal memory B cells were expanded during AHI and then declined after ART administration. These memory B cell changes were not detected in the periphery following ART (Fig. 2).

Early ART prevents peripheral B cell loss

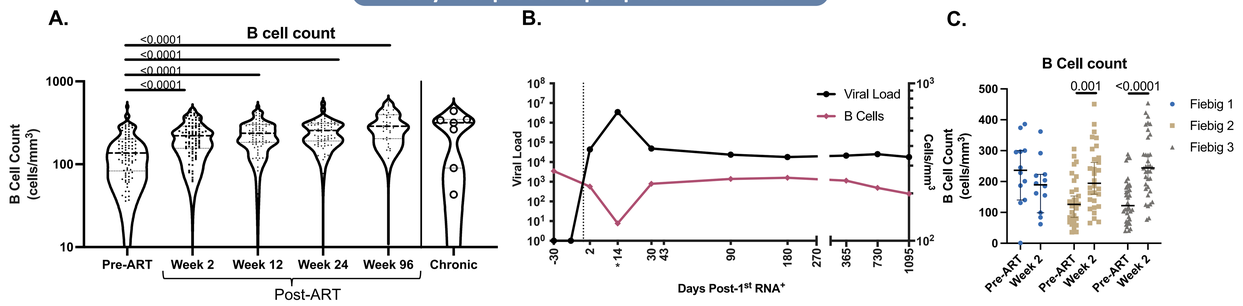

Figure 1. Longitudinal B cell count changes after ART initiation (A) B cell counts (cells/mm3) at indicated weeks post-viremia are shown prior to and during ART therapy. PLWH treated during chronic infection are also shown. EDTA-anticoagulated samples of whole blood were analyzed with the use of the BD Multitest on a FACSCalibur flow cytometer to determine CD19+ B cell counts. Thick and thin dotted lines represent the median and upper and lower quartile of values, respectively. The p-values indicate a significant change in the B cell counts compared to the week 0 time point (Wilcoxon signed-rank). **(B)** Median VL (copies/mL, left axis) and B cell counts (cells/mm3, right axis) of 73 ART-naive participants are shown. Days indicate the number of days since the first positive test for HIV-1 RNA. Day -30 represents all pre-infection samples. B cell counts decrease at peak VL time point, day 14, highlighted with an asterisk. **(C)** B cell counts of donors captured in Fiebig 1-3 at week 0 and week 2 post-ART initiation are indicated. Median, 95% confidence intervals, and p-values by Mann-Whitney are shown. B cell counts significantly increase by 2 weeks post-ART initiation compared to time of first AHI diagnosis (Week 0, **A**), and are significantly higher than untreated PLWH at a similar time post-viremia **(B)**. There are no significant changes in B cell counts of donors captured and treated in Fiebig stage 1 **(C)**, indicating that early treatment prevents B cell loss.

Modulation to the frequencies of peripheral and mucosal B cell phenotypes in the presence or absence of treatment

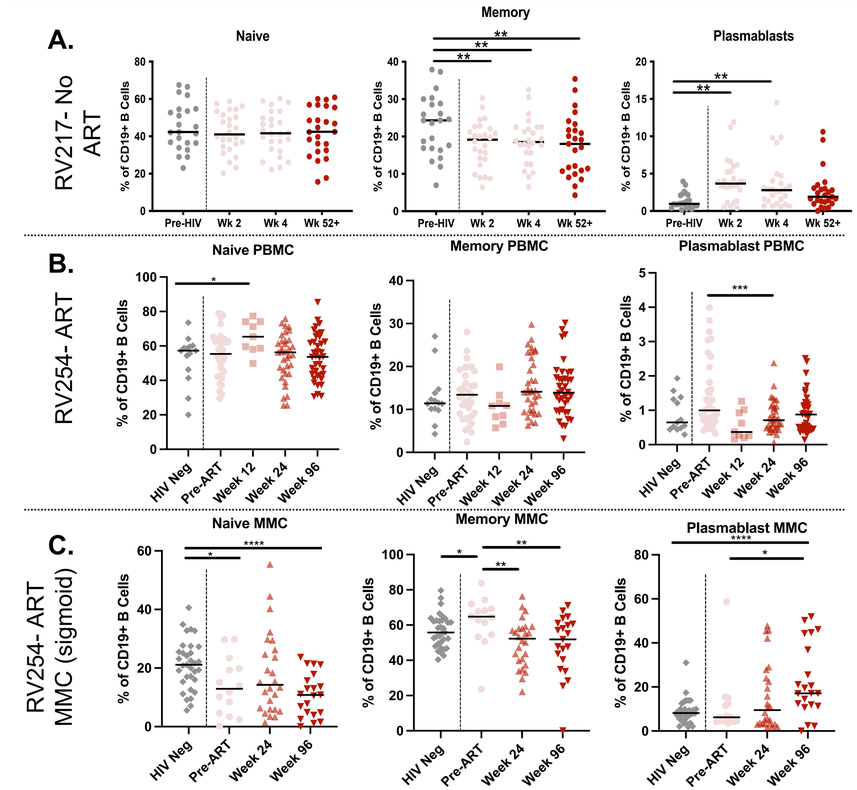

Figure 2. Longitudinal B cell phenotype changes in peripheral and mucosal samples in the presence or absence of therapy Flow cytometry was performed on **(A)** PBMCs from RV217 therapy naïve donors, **(B)** PBMCs from RV254 treated donors, or **(C)** sigmoid biopsies from RV254 treated donors. RV217 timepoints included pre-viremic samples whereas RV254 donor B cell phenotypes were compared to samples from people without HIV from the same region. B cell subsets within the peripheral blood were defined by the expression of CD20, IgD, CD38, and CD27 on CD19+ B cells by increasing levels of differentiation: naive (CD20+/CD27-/IgD+), memory (CD20+/CD27+/IgD-), and plasmablasts, terminally differentiated B cells that secrete immunoglobulin, (CD20-/ CD27+/CD38+). An asterisk (*) above a time point indicates a significant change in the frequency of that B cell phenotype. Wilcoxon signed-rank test was used when comparing matched donors with longitudinal time points and the Mann-Whitney test was used when comparing non-matched donors. Frequencies of peripheral and mucosal memory B cells decrease while plasmablast populations increase in the absence of treatment **(A)**. Memory populations are comparable to HIV negative frequencies in therapy treated peripheral samples **(B)**, however, naive B cell subsets decrease while plasmablast populations increase in the mucosa **(C)**, suggesting ongoing B cell phenotype modulations and, potentially, responses to HIV-1 infection in the presence of therapy.

CONCLUSIONS

- Early ART administration **maintained similar peripheral B cell phenotypes** compared to healthy donors whereas later treatment resulted in **increased B cell activation and differentiation**, likely due to increased antigen load.
- The maintenance of peripheral B cell phenotypes and B cell counts after early ART treatment, especially within Fiebig I captured PLWH, underscores the **benefits of early diagnosis and treatment**.
- **B cell population changes in the sigmoid mucosa** were still detected despite early ART administration, suggesting early B cell responses to HIV in mucosal compartments that can potentially be harnessed in treatment or vaccination strategies aimed at PLWH.
- Therapy strategies can be improved to target and maintain mucosal B cell phenotypes.

MAIN FINDING

Early ART administration retains peripheral B cell counts and phenotypes similar to healthy donors, however B cell population changes occur in sigmoid mucosa despite early ART administration.

ADDITIONAL KEY INFORMATION

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DISCLAIMER
The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army, the Department of Defense, the National Institutes of Health, the Department of Health and Human Services, or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. The investigators have adhered to the policies for protection of human subjects as prescribed in AR-70-25.

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