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Early ART Partly Reduces HIV-Induced Mucosal B-Cell Hyperactivation

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ABSTRACT

HIV-induced immune activation is a hallmark of HIV pathogenesis and disease progression. This includes hyperactivation of B cells with increased differentiation of B cells to plasmablasts (PB) and a decrease in memory B cells (B_{Mem}). We evaluated the effects of antiretroviral therapy (ART) initiated during acute HIV infection (AHI) on mucosal B cells.

Individuals at Fiebig stages I to III of AHI, underwent sigmoid biopsy at the time of HIV diagnosis (AHI; n=14) and 24 months post ART initiation (n=18). Matched HIV-uninfected (HIV-; n=33) and individuals who had initiated ART during chronic HIV infection (CHI - 12 to 36 months post ART initiation; n=6) served as controls. Mucosal mononuclear cells were isolated and multiparameter flow cytometry performed to determine the frequency of B cell subsets (PB: $Lin^-CD20^{+/-}CD19^+CD27^+CD38^{hi}$; B_{Mem}: $Lin^-CD20^+CD19^+CD27^+CD38^-IgD^-IgM^-$).

Median gut HIV RNA was 3.8 log_{10} copies/mg at AHI diagnosis and undetectable after 24 months of ART in 17/18 participants ($p \leq 0.001$). There was no difference observed in the frequency of PB and B_{Mem} between the time of AHI diagnosis and HIV- individuals (PB: 6.3% vs 8.2%, B_{Mem}: 58.0% vs 53.7%, respectively). However, 24 months post ART initiation a significant increase in the frequency of PB to 17.1% was observed compared to time of AHI diagnosis (6.3%; $p \leq 0.01$) and compared to HIV- (8.2%; $p \leq 0.0001$), which was similar to the frequency of PB in CHI (14.8%). The frequency of B_{Mem} decreased from 58.0% during AHI diagnosis to 50.1% at 24 months post ART initiation ($p \leq 0.01$). Following 24 months of ART the frequency of B_{Mem} (50.1%) was lower compared to HIV- (53.7%, p=0.03), but remained significantly higher compared to CHI (35.0%, $p \leq 0.05$). Interestingly, the expression of IgA, the most abundant isotype produced by mucosal PB, was higher in CHI compared to AHI following 24 months of ART (10.8% vs 4.7%, p=0.03), with no increase observed between AHI diagnosis and 24 months post ART initiation (6.1% vs 4.7%).

HIV-related hyperactivation of mucosal B cells was observed despite early ART initiation. However, early ART appears to limit the loss of B_{Mem} compared to CHI as well as prevent increased IgA production by PB. These results provide further insights on mucosal B cell dynamics in HIV infection and another potential mechanism how early ART may limit immune system damage at the mucosal barrier.

BACKGROUND

- HIV-induced immune cell activation is one of the few widely accepted hallmarks of HIV pathogenesis and disease progression
- Hyperactivation of B cells by HIV is characterized by several features, such as hypergammaglobulinaemia, increased polyclonal B cell activation, increased cell turnover, increased expression of activation markers, and increased differentiation of B cell to plasmablasts *(Moir, Nat Rev Immunol, 2009)*
- Initiation of ART within the first 6 months of HIV-infection leads to a better memory B cell response to HIV and non-HIV antigens compared with chronic-treated HIV-infected individuals *(Moir, Immunol, 2018)*
- In SIV, mucosal B cells were associated with delayed HIV acquisition in vaccinated Rhesus Macaques following SIVmac251 rectal challenge *(Tuero, PLoS Pathogens, 2015)*

QUESTIONS

How early during acute HIV-infection (Fiebig I to III) can mucosal B-cell hyperactivation be overserved?

Can initiation of ART during early acute HIV-infection (Fiebig I to III) prevent and/or reverse B-cell hyperactivation?

METHODS

- Thai individuals at Fiebig stage I, II and III enrolled in RV254/SEARCH010 underwent sigmoid biopsies at the time of HIV diagnosis (AHI, n=14), and 6 and 24 months post ART-initiation (n=18)
- Matched HIV-uninfected (HIV-, n=33) and chronic-treated individuals (CHI, n=6) enrolled in RV304/SEARCH013 served as controls
- Mmultiparameter flow cytometry was performed on freshly isolated mucosal mononuclear cells (MMC)
- B cell subsets were determined as follows (Fig 1):
  Plasmablasts (PB): $Lin^-CD20^{+/-}CD19^+CD27^+CD38^{hi}$;
  Memory B cells (B_{mem}): $Lin^-CD20^+CD19^+CD27^+CD38^-IgD^-IgM^-$

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Mucosal B Cells – Gating Strategy

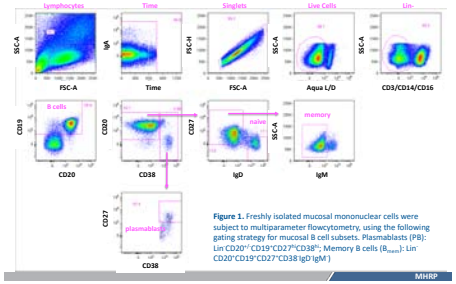


Figure 1. Freshly isolated mucosal mononuclear cells were subject to multiparameter flowcytometry, using the following gating strategy for mucosal B cell subsets. Plasmablasts (PB): $Lin^-CD20^{+/-}CD19^+CD27^{hi}CD38^{hi}$; Memory B cells ($B_{mem}$): $Lin^-CD20^+CD19^+CD27^+CD38^-IgD^-IgM^-$)

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Frequency of Mucosal Memory B Cells Decreases Despite Early ART Initiation

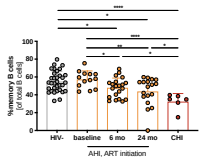


Figure 2. The frequency of mucosal memory B cells at the time of AHI diagnosis was similar compared to HIV-uninfected individuals. However, despite early ART initiation a decline of mucosal B cells was observed 6 and 24 months after ART, but remained significantly higher at any timepoint compared to chronic-treated patients. Memory B cells: $Lin^-CD20^+CD19^+CD27^+CD38^-IgD^-IgM^-$ *p≤0.01, **p≤0.01, ****p≤0.0001

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3

Frequency of Mucosal Plasmablasts Increases Despite Early ART Initiation

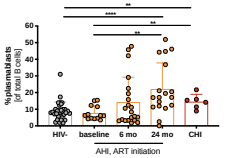


Figure 3. The frequency of mucosal plasmablasts in AHI was similar compared to HIV-uninfected individuals. However, 24 month after ART initiation an increase in the frequency of mucosal plasmablasts could be observed to similar levels seen in chronic-treated patients. Plasmablasts: $Lin^-CD20^{+/-}CD19^+CD27^{hi}CD38^{hi}$, **p≤0.01, ****p≤0.0001

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Frequency of Mucosal IgA⁺ Plasmablasts Remains Comparable to HIV- After Early ART Initiation

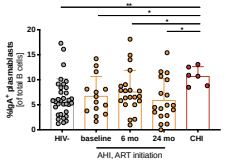


Figure 4. The frequency of mucosal IgA^+ plasmablasts in AHI was comparable to those observed in HIV-uninfected individuals and remained at similar levels 6 and 24 months after ART initiation. In contrast, when ART was initiated during CHI elevated frequencies of IgA^+ plasmablast were observed in those patients. Plasmablasts: $Lin^-CD20^{+/-}CD19^+CD27^{hi}CD38^{hi}$, *p≤0.01, **p≤0.01

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Summary

Table 1. Median frequency of mucosal plasmablasts and memory B cells, and median plasma and gut HIV RNA in HIV-uninfected (HIV-), acutely HIV-infected (AHI) at time of diagnosis and 24 months post ART initiation, and individuals had initiated ART 12 to 36 months ago during chronic HIV infection (CHI).

Median Frequency	HIV- (n=33)	AHI, time of diagnosis (n=14)	AHI, 24 months post ART initiation (n=18)	CHI, ART initiation during CHI (n=6)
plasmablasts [%]	8.2	6.3	17.1[**]	14.8[*]
IgA^+ plasmablasts [%]	5.8	6.1	4.7	10.8[*]
Memory B cells [%]	53.7	58.0	50.1[**]	35.0[**]
Median HIV RNA				
Plasma HIV RNA [log_{10} copies/ml]	NA	6.9 (2.8, 7.7)[a]	1.7[****]	1.9[****] (1.7, 3.0)[a]
Gut HIV RNA [log_{10} copies/mg]	NA	3.8 (1.7, 4.7)[a]	1.8[***] (1.7, 2.4)[a]	1.8[**] (1.7, 2.3)[a]

All data are median (interquartile range). All comparisons were made to AHI, time of diagnosis: *p≤0.05, **p≤0.01, ***p≤0.001; ****p≤0.0001; [a]Range; NA: Not Applicable

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CONCLUSIONS

- During early AHI frequencies of memory B cells and plasmablasts are similar to HIV-uninfected individuals, with no differences between Fiebig stages
- HIV-related hyperactivation of mucosal B cells was observed despite early ART initiation as indicated by the continuous increase in the frequency of mucosal plasmablasts.
- Early ART appears to limit the loss of memory B cells compared to chronic-treated patients as well as prevent increased IgA production by PB.

These results provide further insights on mucosal B cell dynamics in HIV infection and another potential mechanism how early ART may limit immune system damage at the mucosal barrier.

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25. This work was supported by a cooperative agreement (W81XWH-11-2-0174) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DoD).