Persistently Activated CD27+CD80+ B Cells Following Antiretroviral Therapy (ART) Correlate With Macrophage Activation

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ABSTRACT

Background: Antiretroviral therapy (ART) and control of HIV replication does not fully restore perturbations within resting (CD19+CD27+CD21+) and activated (CD19+CD27+CD80+) memory B cell compartments. This study was based on the hypothesis that ongoing macrophage activation and HIV-associated inflammation contributes to B cell abnormalities.

Methodology: Longitudinal cellular and soluble plasma markers for inflammation were evaluated in 43 healthy controls (HC) and 17 behaviorally-infected HIV-1+ subjects ages 19-25. Multicolor flow cytometry analysis of B cell subsets was performed, and assessment of markers of macrophage (sCD163 and sCD14) and lymphocyte (sCD27) activation were measured by ELISA. Assays were performed prior to and at 24 and 48 weeks following ART. All HIV-1 subjects suppressed HIV to <50 copies/ml. Healthy controls and HIV+ treated subjects were also compared pair-wise and longitudinally at study entry (prior to ART), 24 weeks and 48 weeks on therapy by non-parametric rank sum.

Results: Compared to HC, percent of resting memory B cells were lower and activated B cells were higher among HIV+ subjects at all time points before and after ART [22.6 ± 8.1% (HC) versus 16.4 ± 6.7% (HIV+ at 48 weeks) for resting memory, p=0.0081, and 11.5 ± 5.3% (HC) versus 15.2 ± 5.1% (HIV+ at 48 weeks), for activated B cells, p=0.0415, Mann-Whitney]. Control of viral replication failed to decrease the proportions of activated B cells or increase memory B cells after 48 weeks on therapy. Compared to HC, sCD14, sCD27, and sCD163 all remained significantly elevated in HIV+ subjects at 48 weeks following therapy (p=0.0027, p<0.0001, p=0.0142, respectively, Mann-Whitney). Regression analysis revealed a positive correlation between the decrease in resting memory B cells and levels of sCD14 at 48 weeks on therapy (r=0.51, p=0.038, Spearman test) but sCD163 and sCD27 did not correlate with the extent of B cell activation. Results also showed a positive correlation between the increase in activated B cells and the elevation of sCD14 24 weeks following therapy (r=0.54, 0.028, Spearman).

Conclusions: Elevated levels of sCD14, a biomarker of macrophage activation, is the result of LPS binding to TLR4. Our results show that chronic B cell activation also reflects ongoing inflammation due to microbial translocation that may contribute to ongoing B cell dysfunction, even in HIV-infected subjects who control viral replication with ART.

INTRODUCTION

Although antiretroviral therapy (ART) greatly improves the prognosis of HIV-infected individuals, control of viral replication still fails to completely restore functional immunity. There is evidence that defective B cell responses remain following ART, including hyperactivation and dysregulation of some subsets (Klatt 2013). In HIV infection, there is also a massive loss of T cells in mucosal tissues, including GALT or Peyer's patches in the gut. This immune depletion leads to a breakdown of tight junctions that results in microbial products such as LPS reaching the circulation. These products can activate immune cell receptors including TLR4 on macrophages, causing systemic inflammation. Numerous soluble factors can be measured in plasma as indicators of macrophage activation (sCD14, sCD163), lymphocyte activation (sCD27, IFNγ, IL-2) and even vascular inflammation (sVCAM-1, CRP, MPO). The goal of our study is to clarify whether the ongoing systemic inflammation in HIV contributes to B cell abnormalities.

STUDY DESIGN

Study Population: Our cohort included 43 HIV- healthy controls (HC) and 17 behaviorally-infected HIV+ youth ages 18-25. All HIV+ subjects had a CD4 >350 at study entry prior to therapy. Following early treatment with HAART, they all suppressed virus to <50 copies/ul and had stable CD4 >350 throughout 48 weeks of therapy. The HIV-uninfected cohort is balanced for age and gender.

Population	Age, median (range)	Gender, % Female/% Male	Race, n (%)			
			Black, non-hispanic	White, non-hispanic	Hispanic	Other
HIV Negative (n=43)	22 (18-25)	7/93	29 (67)	14 (33)	0 (0)	0 (0)
HIV Positive (n=17)	21 (19-23)	6/94	8 (47)	0 (0)	6 (35)	3 (18)

B Cell Analysis: Whole blood samples were analyzed by multiparameter flow cytometry of B cells using CD19+ as a pan-B cell marker. CD27 (TNF receptor) and CD21 (complement C3d or EBV receptor) were used to further characterize the memory subsets, and CD80 (B7-1) was used as a marker of B cell activation. B cells were analyzed longitudinally and pair-wise at study entry, 24 weeks and 48 weeks following ART initiation, and also compared to HIV negative controls.

Soluble Factor Analysis: Plasma samples were analyzed by ELISA for assessment of macrophage activation (sCD14, sCD163) and lymphocyte activation (sCD27) at entry, 24 weeks and 48 weeks following ART. Outcomes were analyzed longitudinally and pair-wise and also compared to HIV negative controls.

RESULTS

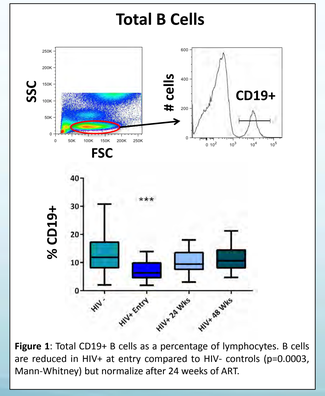


Figure 1: Total CD19+ B cells as a percentage of lymphocytes. B cells are reduced in HIV+ at entry compared to HIV- controls (p=0.0003, Mann-Whitney) but normalize after 24 weeks of ART.

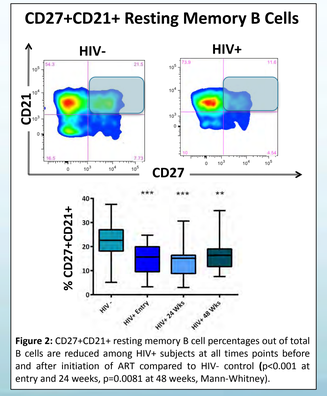


Figure 2: CD27+CD21+ resting memory B cell percentages out of total B cells are reduced among HIV+ subjects at all times points before and after initiation of ART compared to HIV- control (p<0.001 at entry and 24 weeks, p=0.0081 at 48 weeks, Mann-Whitney).

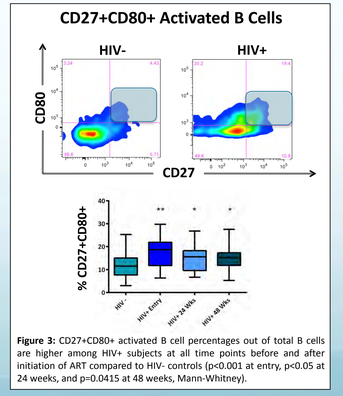


Figure 3: CD27+CD80+ activated B cell percentages out of total B cells are higher among HIV+ subjects at all time points before and after initiation of ART compared to HIV- controls (p<0.001 at entry, p<0.05 at 24 weeks, and p=0.0415 at 48 weeks, Mann-Whitney).

RESULTS

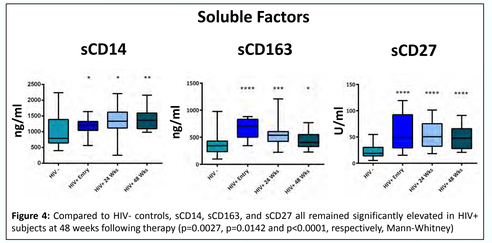


Figure 4: Compared to HIV- controls, sCD14, sCD163, and sCD27 all remained significantly elevated in HIV+ subjects at 48 weeks following therapy (p=0.0027, p=0.0142 and p<0.0001, respectively, Mann-Whitney)

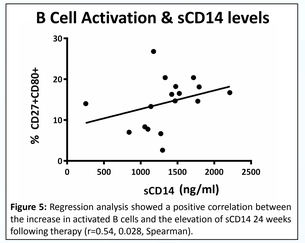


Figure 5: Regression analysis showed a positive correlation between the increase in activated B cells and the elevation of sCD14 24 weeks following therapy (r=0.54, 0.028, Spearman).

CONCLUSIONS

Following initiation of ART, total B cell percentages (CD19+) normalized to values of HIV- controls, however the CD27+CD21+ resting memory subset remained suppressed and CD27+CD80+ activated B cells remained elevated following 48 weeks of ART and despite undetectable viremia. Soluble factors sCD14, sCD163 (macrophage activation) and sCD27 (lymphocyte activation) also remained elevated following 48 weeks of ART. A positive correlation was observed between the increase in activated B cells and the elevation of sCD14 24 weeks following therapy. This data suggests that there is a relationship between the B cell activation that remains despite ART which may be the result of chronic HIV-associated inflammation. Macrophage activation occurring due to microbial translocation and subsequent activation of TLR4 by LPS may be driving the persistent B cell activation that is sustained in the absence of detectable virus. Further correlation analysis using additional soluble factors and B cell markers is required to further clarify this association.

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