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Effect of Antiretroviral Therapy on the Memory and Activation Profiles of B Cells in HIV-Infected African Women

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Human immunodeficiency virus infection induces a wide range of effects in B cells, including skewed memory cell differentiation, compromised B cell function, and hypergammaglobulinemia. However, data on the extent to which these B cell abnormalities can be reversed by antiretroviral therapy (ART) are limited. To investigate the effect of ART on B cells, the activation (CD86) and differentiation (IgD, CD27, and CD38) profiles of B cells were measured longitudinally in 19 HIV-infected individuals before (median, 2 mo) and after ART initiation (median, 12 mo) and compared with 19 age-matched HIV-uninfected individuals using flow cytometry. Twelve months of ART restored the typical distribution of B cell subsets, increasing the proportion of naive B cells ($CD27^-IgD^+CD38^-$) and concomitantly decreasing the immature transitional ($CD27^-IgD^+CD38^+$), unswitched memory ($CD27^+IgD^+CD38^-$), switched memory ($CD27^+IgD^-CD38^-$ or $CD27^-IgD^-CD38^-$), and plasmablast ($CD27^+IgD^-CD38^{high}$) subsets. However, B cell activation was only partially normalized post-ART, with the frequency of activated B cells ($CD86^+CD40^+$) reduced compared with pre-ART levels ($p = 0.0001$), but remaining significantly higher compared with HIV-uninfected individuals ($p = 0.0001$). Interestingly, unlike for T cell activation profiles, the extent of B cell activation prior to ART did not correlate with HIV plasma viral load, but positively associated with plasma sCD14 levels ($p = 0.01$, $r = 0.58$). Overall, ART partially normalizes the skewed B cell profiles induced by HIV, with some activation persisting. Understanding the effects of HIV on B cell dysfunction and restoration following ART may provide important insights into the mechanisms of HIV pathogenesis. ***The Journal of Immunology*****, 2017, 198: 1220–1228.**

Systemic immune hyperactivation is a hallmark of HIV infection, affecting a range of immune cells, including both T cells and B cells (1). Multiple B cell defects have been reported in HIV-infected individuals, including alteration in the distribution of B cell memory subsets, with the accumulation of differentiated B cells (2–5), excessive B cell activation (6, 7), and increased cell turnover (8). These B cell perturbations lead to functional abnormalities, as demonstrated by hypergammaglobulinemia, decreased B cell responsiveness to both T cell-dependent and T cell-independent Ags (9–11), and compromised responses to vaccination (9, 12, 13).

The specific mechanisms contributing to B cell abnormalities are only partially known, and multiple factors may account for their dysfunction. HIV-driven alteration of the cytokine and chemokine environment has been described as a source of B cell dysfunction (5, 14–16); and it has also been proposed that specific HIV proteins may have a direct effect on B cells (17, 18). Several studies, performed mostly in cross-sectional Caucasian cohorts, have investigated the effect of antiretroviral therapy (ART) on B cells, reporting that suppressive ART can partially or completely normalize B cell phenotypic defects, as shown by the replenishment of naive B cells (19–22), contraction of activated B cells (21–25), and increase in B cell survival potential (26). It is still uncertain, however, whether normalization of B cell memory subsets results in improved B cell immune responses to Ags, including influenza, measles, pneumococcus, and hepatitis B (6, 10, 11, 27, 28).

There is a paucity of published studies on female and African populations with regard to B cell activation and restoration of B cell immunity following successful treatment of HIV (29, 30). There are cogent reasons to believe there may be differences in African compared with Caucasian cohorts. African cohorts have demonstrated higher baseline levels of T cell activation, significantly different T cell memory differentiation profiles (31, 32), and consistently weaker cellular and humoral reactivity to some vaccines (33, 34). A variety of factors may influence immune activation and therefore normalization of immune profiles after ART,

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Abbreviations used in this article: ART, antiretroviral therapy; CAPRISA, Centre for the AIDS Program of Research in South Africa; IQR, interquartile range; LDL, lower detection limit.

such as genetic, gender, and environmental differences, the latter including higher antigenic exposure, diet, and gut microbiota. Furthermore, a variety of sex-specific differences in the response to infections have been described. Women have higher levels of immune activation and faster progression of HIV disease than men with the same viral load (35). These effects have been attributed to estrogen receptor signaling and/or differences in expression of key X-chromosome-expressed immune regulators, such as TLR and CD40L (36). Additional factors such as HIV strains, treatment regimens, and delayed access to HIV treatment could result in distinct outcomes with respect to immunity after ART.

Thus, in this study, to define the extent to which ART restores the B cell phenotype, we measured the memory differentiation and activation profiles of B cells longitudinally in chronically HIV-infected African women before and 12 mo after ART initiation, and compared these profiles to age- and sex-matched HIV-uninfected individuals.

Materials and Methods

Description of study participants

Study participants consisted of 19 women from the Centre for the AIDS Program of Research in South Africa (CAPRISA) 002 HIV acute infection cohort in KwaZulu-Natal, as previously described (37, 38). Peripheral blood samples were obtained at two time points, during chronic infection pre-ART initiation, and post-ART initiation. With respect to ART regimens, 15 of the 19 participants were taking current standard first-line therapy (TDF/3TC/EFV or TDF/FTC/EFV), and four took one of D4T/3TC/EFV, D4T/3TC/NVP, AZT/3TC/NVP, or AZT/3TC/LPV/r. One participant (CAP255) switched ART regimens during the study period (D4T/3TC/EFV to AZT/3TC/EFV at month 10). No participants had active tuberculosis during the study period, or exhibited any immune reconstitution inflammatory syndrome upon HIV treatment. An additional 19 HIV-uninfected women who were matched for age and ethnicity were studied, from the CAPRISA 004 1% tenofovir microbicide gel trial (39). These women were either in the preintervention or placebo arm of the trial. For HIV-infected individuals, either a prospective RNA positive/Ab negative reading or the midpoint between the last Ab negative test and the first Ab positive ELISA test were used to determine the time postinfection. Highly active ART was given according to the South African national HIV treatment guidelines (at a CD4 count of $<$200 cells/mm^3 prior to October 2012; $<$350 cells/mm^3 until March 2015). Ethical approval for the study was obtained from the Research Ethics Committees at the University of KwaZulu-Natal and the University of Cape Town. All participants provided written informed consent prior to participating in the study.

Measurements of HIV plasma viral load and CD4 counts

Plasma HIV viral load and CD4 count were assessed at each study visit. Over the course of the study, the viral load PCR assay switched from Roche AMPLICOR HIV-1 monitor test version 1.5 (lower detection limit [LDL] of 400 RNA copies/ml) to Roche Taqman version 1.0 in June 2010 (LDL 40 RNA copies/ml), and then to Roche Taqman version 2.0 in January 2012 (LDL 20 RNA copies/ml). The FACSCalibur TruCOUNT method (BD Biosciences) was used to measure blood absolute CD4$^+$ and CD8$^+$ T cell counts.

Sample processing

PBMC were isolated by Ficoll-Hypaque (Amersham Pharmacia) density gradient centrifugation and cryopreserved in freezing media (heat-inactivated FCS; Invitrogen) containing 10% DMSO (Sigma-Aldrich). Cells were stored in liquid nitrogen until use. Cryopreserved PBMC were thawed and rested in R10 (RPMI 1640 plus 10% heat-inactivated FCS and 50 U/ml of penicillin-streptomycin) at 37˚C with 5% CO_2 for 3 h before staining.

Abs, surface, and intracellular staining

The following Abs were used in three different staining panels: CD19 ECD (J3-119), CD27 PE-Cy5 (1A4CD27; both Beckman Coulter), IgD APC-Cy7 (IA6-2), CD10 BV605 (HI10a), CD21 PE-Cy7 (Bu32), CD40 PerCP-Cy5.5 (5C3; all BioLegend), CD38 APC (HIT2), CD86 PE (IT2.2), CD3 PE-Cy7 (SK7), HLA-DR APC-Cy7 (L243; all BD Biosciences), CD4 PE-Cy5.5 (S3.5), CD8 Qdot-705 (3B5), CD19 Pacific Blue (SJ25-CI), CD14 Pacific Blue (Tük4), CD3 Pacific Blue (UCHT1), Ki67 FITC (7B11; all Invitrogen), and a violet viability reactive dye (Vivid; Molecular Probes). All Abs were titrated prior to use to obtain optimal titers for staining. Briefly, PBMC were stained with Vivid, then labeled with Abs against surface markers, fixed, permeabilized, and subsequently stained intracellularly with Ki67. Cells were then resuspended in 1× CellFix (BD Biosciences) and kept at 4˚C until acquisition. Samples were acquired on a BD Fortessa using FACSDiva software and analyzed using FlowJo (version 9.9.3; TreeStar). The gating strategy to identify B cell subsets is shown in Supplemental Fig. 1.

ELISAs

Plasma samples were used to measure soluble CD14 (sCD14; a marker of monocyte/macrophage activation) from the same women pre- and post-ART. One participant was not included in this analysis due to the sample being unavailable. Plasma (n = 18) was tested in duplicate using commercially available ELISA kits according to the manufacturer's protocol (R&D Systems). In addition, 30 plasma samples from HIV-uninfected women from the same cohort were included. Samples were diluted 1:200, data were collected using a SpectraMax Plus reader (Molecular Devices), and point-to-point curve fits were used to calculate sCD14 concentrations from the standard curves. Data were analyzed using SoftMax Pro software (Version 3.2.1; Molecular Devices).

Statistical analyses

Statistical analyses were performed using GraphPad Prism (version 5.0). The Mann–Whitney U test and the Wilcoxon Signed Rank test were used for unmatched and paired samples, respectively. Correlations between the different groups were determined by the non-parametric Spearman Rank test. A p value $<$0.05 was considered statistically significant.

Results

Effect of ART on the memory differentiation and activation of B cells

To define the extent to which ART normalizes HIV-induced alteration of B cells, we first determined the distribution of B cell subsets in 19 HIV-infected individuals before and 1 y after ART initiation, and compared it to 19 HIV-uninfected matched controls. Pre-ART samples were obtained during chronic infection at a median of 4.9 y (interquartile range [IQR]: 2.8–5.8 y) after the estimated date of HIV infection, and a median of 1.5 mo (IQR: 0.05–2.7) prior to starting ART. The median plasma viral load at this time was 34,325 HIV RNA copies/ml (IQR: 7,997–78,573), and the median CD4 count was 314 cells/mm^3 (IQR: 260–369) (Table I). After a median of 12 mo of treatment (IQR: 12–13 mo), all individuals exhibited viral suppression (p = 0.0001), the majority to $<$40 copies/ml, and an increase in both absolute CD4 count and CD4/CD8 ratio (both p = 0.0001) (Table I, data not shown).

Fig. 1A shows representative flow plots of each B cell subset from one HIV-uninfected and one HIV-infected individual pre- and post-ART. Based on the expression of CD27, IgD, and CD38, six B cell subsets were identified, namely immature transitional B cells (CD27$^-$IgD$^+$CD38$^+$), naive B cells (CD27$^-$IgD$^+$CD38$^-$), unswitched memory (CD27$^+$IgD$^+$CD38$^-$), CD27$^+$ switched memory B cells (IgD$^-$CD38$^-$), CD27$^-$ switched memory B cells (IgD$^-$CD38$^-$), and plasmablasts (CD27$^+$IgD$^-$CD38^{high}). There was a marked skewing of B cell subsets in chronic HIV infection prior to ART (Fig. 1B). When compared with HIV-uninfected participants, HIV-infected individuals were characterized by significantly higher frequencies of immature transitional B cells, unswitched memory B cells, and plasmablasts (median: 9.3% [IQR: 6.3–13.3] versus 14.8% [IQR: 10.6–20]; 1.5% [IQR: 1.2–1.7] versus 2.3% [IQR: 2–5.8] and 0.44% [IQR: 0.3–1] versus 2.1% [IQR: 1.4–4], respectively), and significantly lower proportions of naive B cells (median: 65.8% [IQR: 60–72.1] versus 46.9% [IQR: 39.7–55.7]) (Fig. 1B). Additionally, the frequencies of switched memory B cells expressing CD27 or not were comparable in HIV-uninfected and ART naive HIV-infected individuals

Table I. Clinical parameters of study participants

	HIV-Infected (Pre-ART)	HIV-Infected (Post-ART)	*p* Value
Sample size	19	19 (paired)	
CD4 count (cells/mm^3)[a]	314 (260–369)	629 (514–696)	$p = 0.0001$
CD4/CD8 ratio[a]	0.23 (0.18–0.33)	0.61 (0.48–0.74)	$p = 0.0001$
Viral load (RNA copies/ml)[a]	34,325 (7,997–78,573)	<20 (<20–39)	$p = 0.0001$
Duration of ART (months)[a]	NA	12 (12–13)	

[a]Median and interquartile range.
NA, not applicable.

(~8 and ~12%, respectively). It is worth mentioning that the frequency of unswitched memory B cells in our healthy study population was ~7 times lower when compared with a previous study reporting that the proportion of unswitched memory was comparable to switched memory (representing ~15% of total B cells) in a predominantly male cohort from London (11). Following 12 mo of ART, the frequency of immature transitional, naive and unswitched memory B cells were normalized, returning to levels comparable to those observed in HIV-uninfected individuals (Fig. 1B). The frequency of plasmablasts was also reduced in response to ART ($p = 0.004$), but remained significantly elevated compared with the HIV-uninfected group. To further define the restoration dynamics of B cell subsets upon ART, we compared the fold change in their frequencies pre- and post-ART. After 1 y of ART, only naive B cells displayed an overall expansion (median 1.3 fold [IQR: 1.2–1.5]); among the subsets decreasing upon ART, plasmablasts exhibited the largest contraction, with a median fold change of 0.38 (IQR: 0.22–0.72). The contraction of other B cells subsets was more moderate with a median fold change of 0.51 (IQR: 0.39–0.92) for unswitched memory B cells, 0.65 (IQR: 0.37–0.77) for immature transitional B cells, 0.79 (IQR: 0.5–1) for CD27$^+$ switched memory B cells and 0.87 (IQR: 0.69–1) CD27$^-$ switched memory B cells (Fig. 1C). Although ART had a predominant effect on reducing the frequency of plasmablasts (~60% reduction), this was not sufficient for their normalization with respect to HIV-uninfected individuals.

Alternative B cell markers such as CD27, CD10, and CD21 have been used to delineate B cell memory subsets (40), allowing for the identification of three additional B cell memory subsets, namely tissue-like memory (CD10$^-$CD21$^-$CD27$^-$), resting memory (CD21$^+$CD27dim), and activated memory (CD10$^-$CD21$^-$CD27dim) B cells (Supplemental Fig. 1B). The expression of CD10 and CD21 on B cell subsets is shown in Supplemental Fig. 2. Thus, to validate our data, we defined the evolution of B cell memory subpopulations pre- and post-ART using this set of markers in 10 of the HIV-infected individuals from our study (Supplemental Fig. 3). ART led to a significant reduction in the frequencies of tissue-like memory, activated memory B cells, and plasmablasts, and an increase in the frequencies of naive and resting memory B cells. These observations confirm the data described above, and are in line with published data (21). Of note, using this alternative gating strategy, we found no significant difference in the frequencies of immature transitional B cells between the two time points. This may be due to the small sample size (a subset of $n = 10$) used for this set of markers, because further analysis of the same individuals using IgD, CD38, and CD27 also showed no significant difference in immature transitional B cells (data not shown).

The effects of ART on B cell activation and proliferation were also evaluated. Fig. 2A shows representative flow plots of B cell activation, measured as the frequency of B cells coexpressing CD86 and CD40, and proliferation (Ki67 expression) from one HIV-uninfected and one HIV-infected individual pre- and post-ART. The frequency of activated B cells (CD86$^+$CD40$^+$) was significantly higher in HIV-infected subjects prior to ART compared with HIV-uninfected participants (median: 14% [IQR: 9.2–15.6] versus 1.7% [IQR: 1.3–2.3]; Fig. 2B). In response to ART, the frequency of activated B cells was substantially reduced but remained significantly higher than in HIV-uninfected participants (Fig. 2B). It is important to mention that during HIV infection, activated B cells were mostly confined to switched memory cells (i.e., IgD$^-$ B cells), with a median of 25% (IQR: 19.6–32) of switched cells coexpressing CD40 and CD86. On the contrary, the activation level of unswitched B cells (i.e., IgD$^+$ B cells) was only marginal, with less than 5% of these cells positive for CD40 and CD86 (median: 4.5% [IQR: 2.4–5]; data not shown).

Similar to activated B cells, there were significantly more Ki67$^+$ B cells in ART-naive HIV-infected individuals compared with HIV-uninfected controls (median: 5% [IQR: 2.9–7.2] versus 1.3% [IQR: 0.9–1.6]) and although ART led to a significant decline in proliferating B cells, they remained elevated compared with the HIV-uninfected group ($p = 0.001$, Fig. 2C). Of note, the frequencies of proliferating (Ki67$^+$) and activated (CD86$^+$CD40$^+$) B cells were positively associated with the frequency of plasmablasts following ART ($p = 0.004$, $r = 0.62$ and $p = 0.003$, $r = 0.63$, respectively, data not shown), suggesting that residual B cell activation and proliferation upon ART may be explained by the persistence of an elevated frequency of plasmablasts, consistent with our findings presented in Fig. 1B.

Overall, our data show that HIV infection skews the B cell memory differentiation profile and causes abnormal B cell activation and proliferation. Defects in B cell memory phenotype were largely normalized within 1 y of ART, whereas B cell activation and proliferative capacities were only partially reduced and longer periods of suppressive therapy may be required for normalization.

B cell defects during HIV infection do not directly associate with HIV viral load

It has been clearly established that hyper-immune activation of T cells during HIV infection is related to viral replication (41) and is partially normalized upon ART (42). This was confirmed in our experimental setting, where we had T cell activation data for 18 of the participants in the current study, as well as 14 additional subjects from the same cohort. The proportion of activated CD4$^+$ T cells (measured by the expression of HLA-DR) was positively associated with plasma viral load ($p = 0.001$, $r = 0.54$; Fig. 3A) in HIV-infected subjects pre-ART. Although 12 mo of ART led to a substantial reduction in the frequency of HLA-DR expression by T cells, T cell activation levels remained significantly higher than in HIV-uninfected subjects (Supplemental Fig. 4). Thus, to determine whether HIV load was also the driving factor for B cell activation, we examined the relationship between B cell phenotype and HIV plasma viral load before ART initiation. As previously

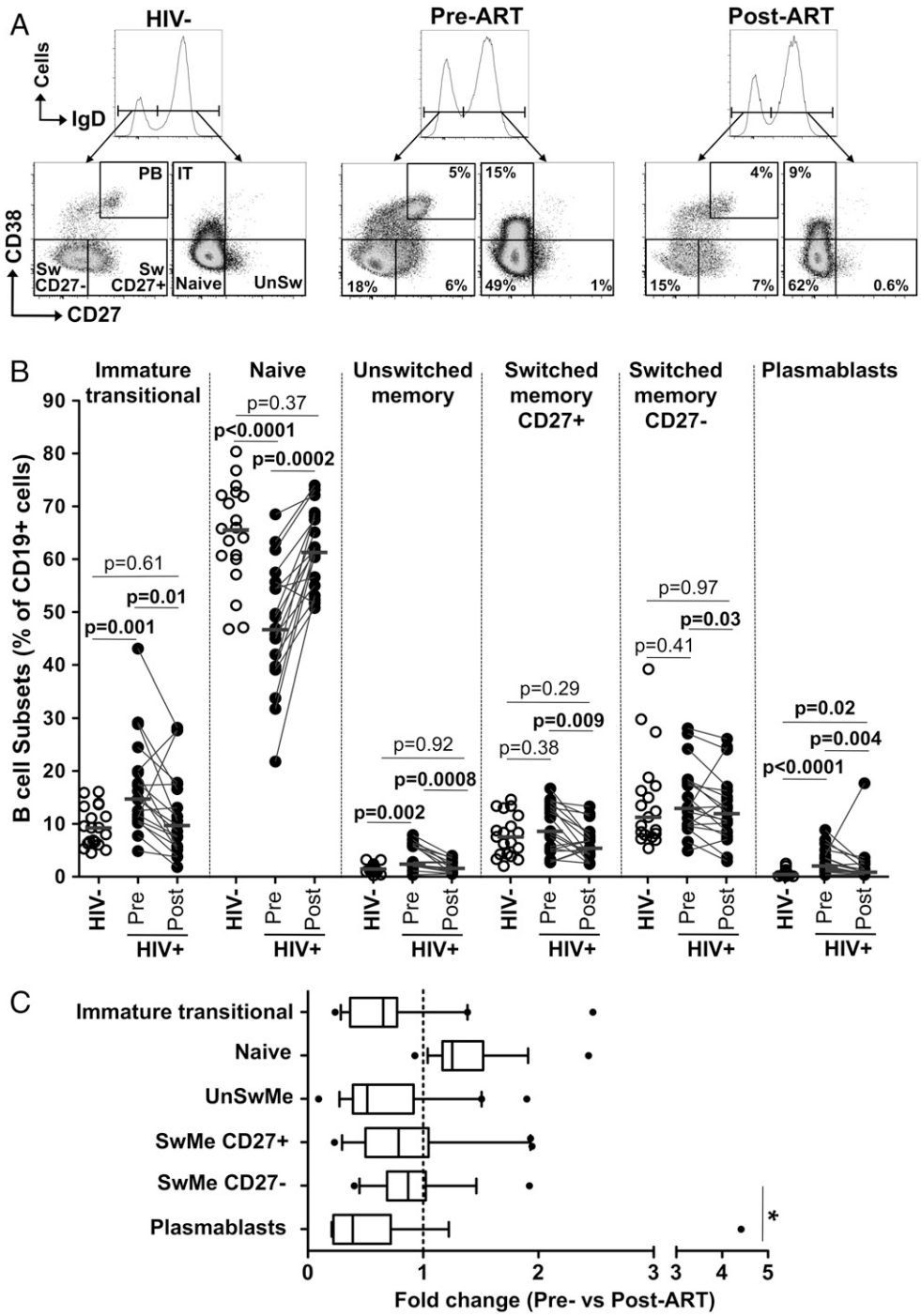

FIGURE 1. Effect of ART on the memory differentiation profile of B cells. (**A**) Representative flow plots of CD27, IgD, and CD38 expression in B cells from one HIV-uninfected and one HIV-infected (pre- and post-ART) individual. Numbers represent the frequencies of each B cell subset, namely immature transitional ($CD27^-IgD^+CD38^+$), naive ($CD27^-IgD^+CD38^-$), Unswitched memory ($CD27^+IgD^+CD38^-$), switched $CD27^+$ memory (IgD^-CD38^-), switched $CD27^-$ memory (IgD^-CD38^-), and plasmablasts ($CD27^+IgD^-CD38^{high}$). (**B**) Frequencies of B cell subsets in HIV-uninfected ($n = 19$; open circles) and HIV-infected pre- and post ART ($n = 19$; closed circles) individuals. Horizontal lines indicate the median. Statistical significance was calculated using a Mann–Whitney *U* test and Wilcoxon Signed Rank for unpaired and paired samples, respectively. (**C**) Fold change in the frequency of B cell subsets over 12 mo of ART. Data are shown as box and whisker (interquartile range) plots. The vertical dotted line indicates no change from the time point prior to ART. Statistical comparisons were calculated using a one-way ANOVA test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

stated, switched memory B cells (i.e., IgD^- B cells) are highly activated during HIV infection, thus we focused our analyses on this particular subset. Surprisingly, unlike for T cells, we found no association between the frequency of activated ($CD86^+CD40^+$) switched memory B cells and HIV viral load ($p = 0.71$, $r = 0.39$; Fig. 3B). Moreover, none of the B cell memory subset frequencies were found to be associated with viral load (data not shown). Finally, the extent of $CD4^+$ or $CD8^+$ T cell activation did not correlate with the level of switched B cell activation ($p = 0.78$, $r = 0.07$; Fig. 3C, data not shown). Together, these results show that HIV differentially affects the T cell and B cell compartments, where B cell activation does not mirror T cell activation and is not directly associated with the levels of HIV. This suggests that additional factors besides HIV replication may contribute to B cell activation.

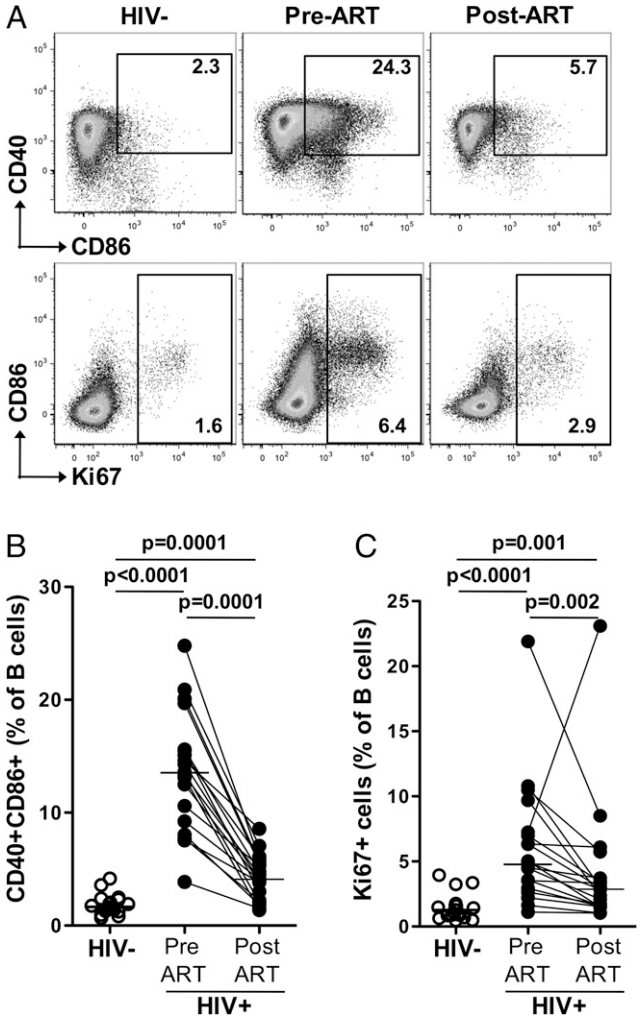

FIGURE 2. Effect of ART on B cell activation and proliferation. (**A**) Representative flow plots of CD40, CD86, and Ki67 expression in B cells from one HIV-uninfected and one HIV-infected (pre- and post-ART) individual. Numbers represent the frequencies of activated ($CD86^+CD40^+$) and proliferating ($Ki67^+$) B cells. (**B**) Frequencies of $CD86^+CD40^+$ B cells in HIV-uninfected ($n = 19$; open circles) and HIV-infected pre- and post-ART ($n = 19$; closed circles) individuals. (**C**) Frequencies of proliferating B cells as measured by Ki67 expression. Horizontal lines represent the median. Statistical significance was calculated using a Mann–Whitney *U* test and Wilcoxon Signed Rank for unpaired and paired samples, respectively.

Additional contributors of B cell defects during HIV infection

The translocation of bacterial products resulting from HIV-induced damage to the gastrointestinal tract is an important contributor of systemic immune activation (43). Therefore, we investigated the relationship between the activation and skewed differentiation profile of B cells and the level of plasma sCD14, a marker of monocyte/macrophage activation, which may be an indicator, in the absence of other infections, of microbial translocation. Prior to ART, plasma sCD14 levels associated negatively with the frequency of naive B cells ($p = 0.036$, $r = -0.50$; data not shown) and positively with the frequency of immature transitional B cells ($p = 0.003$, $r = 0.75$; Fig. 4A), and the frequency of activated ($CD86^+CD40^+$) switched memory B cells ($p = 0.01$, $r = 0.58$; Fig. 4B). Moreover, a positive correlation was also observed between sCD14 levels and the frequency of activated HLA-DR^+ $CD4^+$ T cells ($p = 0.045$, $r = 0.49$; Fig. 4C). It is worth noting that the levels of plasma sCD14 were not significantly changed after 12 mo of ART (Supplemental Fig. 5), and there were no associations with residual B cell activation post-ART (data not shown).

Overall, these data suggest a possible impact of microbial translocation on B cell dysfunction, as suggested by sCD14 levels correlating with HIV-induced B cell skewing and hyperactivation in untreated chronic HIV infection. However, their influence may be relatively minor, because there are consistently elevated levels of sCD14 after ART, when B cell defects are largely reversed. It is clear that a variety of factors may directly or indirectly influence B cell defects during HIV infection.

Discussion

Despite the fact that HIV does not directly infect B cells, the virus causes several B cell abnormalities that contribute to HIV pathogenesis (44). Although these B cell defects may be reverted upon ART, this effect has mostly been studied in cross-sectional Caucasian cohorts. Thus, in this study we assessed longitudinally the impact of ART on the memory and activation profile of B cells in South African adult women ($n = 19$); and compared these profiles to age-matched HIV-uninfected women from the same community ($n = 19$). Our data show that: 1) HIV-induced alterations of B cells did not associate with viral burden but rather correlated with plasma sCD14 concentration; and 2) 12 mo of ART largely normalized the distribution of B cell memory subsets whereas activation of B cells was only partially reduced.

HIV induces multiple defects in B cells, altering their memory and activation profiles and function (3, 5, 40, 45). In our experimental setting, we showed that in HIV-infected African women B cells were skewed toward a more differentiated memory phenotype (with the accumulation of plasmablasts and the reduction of naive B cells) and were hyperactivated (as measured by CD86 expression) when compared with HIV-uninfected persons. These observations are in accordance with previous studies performed primarily in male Caucasian populations (3, 20, 21, 6, 45, 46), highlighting that similar abnormalities occur in African women. To date, the drivers of B cell alterations during HIV infection have not been fully elucidated. Even though HIV does not directly target B cells for infection, the interaction between HIV and B cells has been previously described, where HIV gp120 can bind to B cell receptors such as CD21, DC-SIGN, and variable H chain 3 (VH3) Ig, resulting in polyclonal B cell activation and Ig production (47). Furthermore, HIV Nef can interact directly with B cells, resulting in suppression of Ig class switching (48, 49). How each of these mechanisms contributes to B cell dysfunction is unknown (40).

It has also been proposed that excessive B cell activation may be driven by proinflammatory cytokines, such as IFN-α, TNF-α, IL-6, and IL-10, molecules commonly up-regulated during HIV-induced systemic inflammation (50). HIV infection also leads to increased production of B cell-specific growth factors such as BLyS/BAFF from macrophages, dendritic cells, and granulocytes (51–53), promoting B cell dysregulation right from the early stages of infection. Additionally, HIV pathogenesis has also been linked to extensive damage to the gastrointestinal tract, leading to the release of bacterial products into the circulation (43). These products may induce aberrant B cell activation via TLR signaling (54), by both direct and indirect means. Stimulation of monocytes by bacterial LPS leads to the release of cytokines that contribute to the inflammatory environment. Additionally, human B cells express a range of TLRs, including high levels of TLR9 (55), and abundant bacterial DNA as a result of HIV-induced microbial translocation (56) may stimulate B cells directly. In fact, TLR9 stimulation of human B cells promotes their activation, proliferation, and the generation of

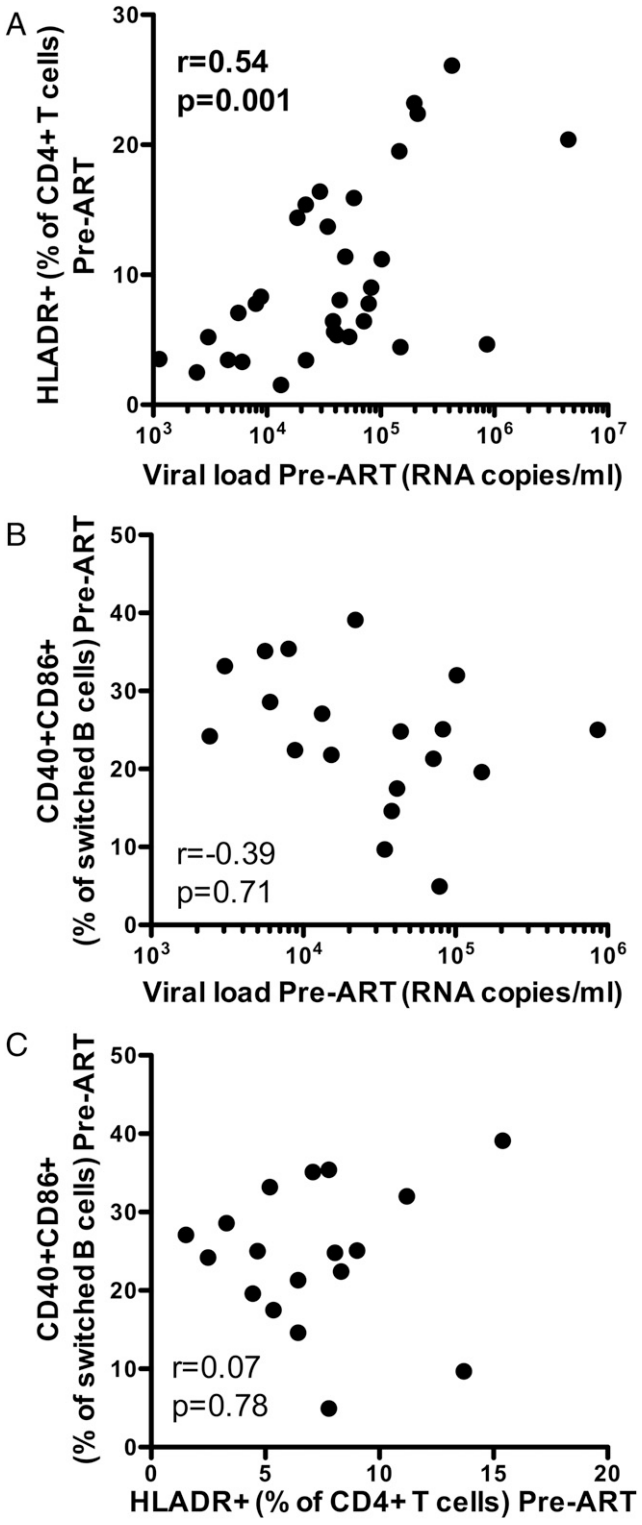

FIGURE 3. Relationship between $CD4^+$ T cell and B cell activation and plasma viral load pre-ART. Correlation of (**A**) the frequency of HLA-DR expressing $CD4^+$ T cells (n = 32) and (**B**) the frequency of $CD40^+CD86^+$ switched memory B cells (n = 19) and plasma viral load in HIV-infected individuals prior to ART initiation. (**C**) Correlation between B cell activation ($CD86^+CD40^+$) and $CD4^+$ T cell activation ($HLA\text{-}DR^+$) in 18 HIV-infected participants before ART. Statistical significance was calculated using a non-parametric Spearman Rank test.

plasmablasts (57, 58). The potential role of microbial products as direct factors fueling B cell hyperactivation is further inferred by the fact that in other chronic diseases leading to gut damage (such as inflammatory bowel disease), B cells exhibit aberrant activation profiles (59).

We found an association pre-ART of B cell activation and differentiation profile with the concentration of plasma sCD14, secreted upon monocyte/macrophage activation by LPS, which may be a surrogate marker of microbial translocation. Interestingly, although there was a relative normalization of B cells post-ART, plasma sCD14 concentration did not decrease overall upon treatment. This phenomenon has been observed in several studies, where sCD14 levels remain elevated even after several years on ART (60–62), and unreversed damage to the gastrointestinal tract has been proposed to account for this. These data confirm that in African women, inflammation persists after ART even when there is a substantial decrease in cellular activation. The absence of any decrease in sCD14 after ART, despite a substantial normalization of B cell profiles, suggests that sCD14 plays only a partial role in contributing to B cell activation. Alternatively, we could speculate that microbial translocation and viral-induced factors might act synergistically to drive B cell activation and skewing, and ART uncouples this effect by suppressing viral replication even in the face of ongoing inflammation. Although we did not find an association of HIV viral load with B cell dysfunction pre-ART, this relationship may not be linear, with a multitude of viral-induced factors influencing B cell activation and differentiation directly and indirectly (63). Overall, this suggests that a combination of inflammatory mediators such as cytokines, B cell growth factors, and microbial products may directly and indirectly stimulate B cell differentiation and proliferation, favoring their functional impairment and exhaustion.

Several studies have shown that ART leads to a near normalization of peripheral B cell memory subsets (19, 20, 22, 64); and the extent of restoration was linked to the timing of ART initiation (21, 65), the duration of treatment, or the age of the patients (22, 66). Very limited data are available on the effects of ART on B cells in African cohorts. To our knowledge, only one cross-sectional study has investigated how ART influences the restoration of B cell numbers in an African adult population (30). In countries disproportionately affected by the HIV pandemic and where access to ART is limited and often started during advanced lymphopenia, it is of importance to define whether HIV treatment can restore B cell profiles to similar levels observed in HIV-uninfected individuals (of note, the CD4 count pretreatment in the participants included in this study was $<$350 cells/mm^3). Our data showed that although 12 mo of ART largely normalized the distribution of memory subsets, B cell activation remained significantly elevated compared with HIV-uninfected individuals. The persistence of these cycling, activated B cells composed mainly of plasmablasts could reflect residual systemic inflammation, despite viral suppression. Indeed, it has been shown that even upon successful ART, inflammation, microbial translocation, and germinal center destruction still persist (43, 67–69). These observations are in line with results reported in Caucasian cohorts (20, 21, 70), showing that the normalization of B cell subsets can be achieved even when treatment is initiated at a late stage of chronic HIV infection.

The limitations of this study include a cohort exclusively composed of women; further studies could test the generalizability of our findings in men. Additionally, we only analyzed B cells from peripheral blood and this may not be representative of B cell profiles within the tissues; it is plausible that B cells residing in the germinal center are differentially affected during HIV infection and that the extent and/or kinetics of their restoration during treatment may differ. For future studies, use of additional phenotypic markers such as CD10 and CD24 could improve the

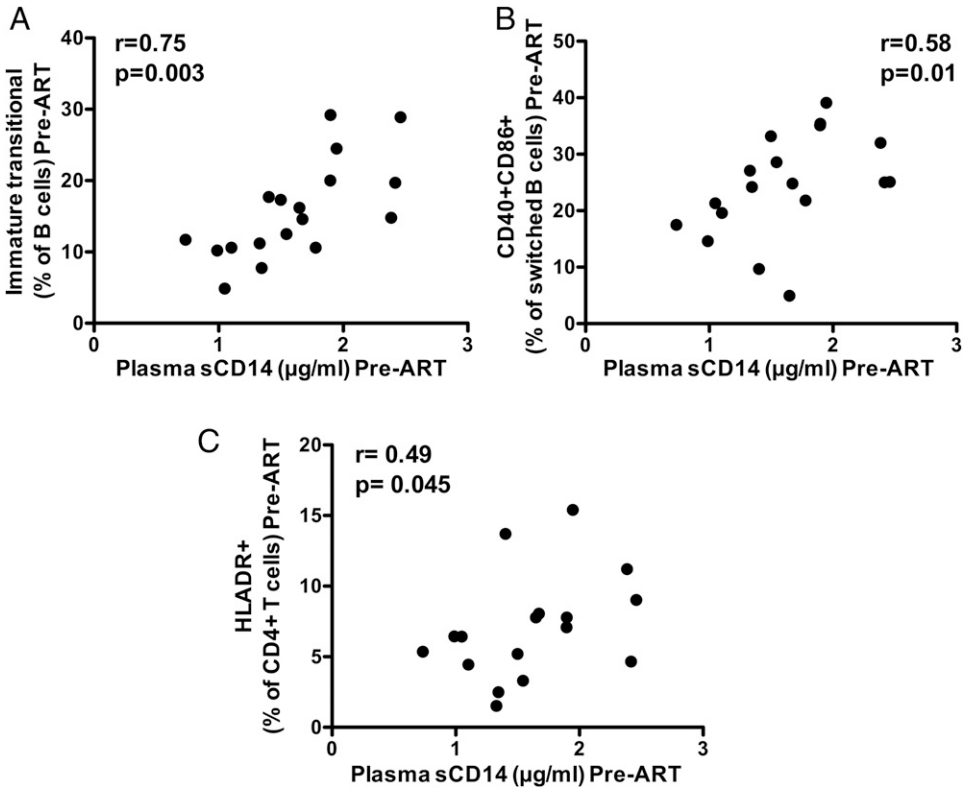

FIGURE 4. Relationship between plasma sCD14 levels and B cell phenotype and $CD4^+$ T cell activation pre-ART. Correlation of (**A**) the frequency of immature transitional B cells, (**B**) the frequency of $CD86^+$ $CD40^+$ switched memory B cells, and (**C**) and the frequency of $HLA\text{-}DR^+$ $CD4^+$ T cells with plasma sCD14 levels in HIV-infected individuals (n = 18 and n = 17, respectively). Statistical significance was calculated using a non-parametric Spearman Rank test.

definition of B cell subsets, in particular immature transitional B cells. Furthermore, we did not examine the relationship of relative normalization of B cell activation and differentiation with restoration of functional B cell immunity, which may not always return (71). Further studies of humoral immunity after ART are warranted in larger African cohorts, by investigation of both memory B cell responses and long-lived plasma cells to a variety of vaccines and infections. This is particularly important if functional B cell immunity is not fully restored and revaccination to certain pathogens may be required. In this respect, early ART initiation, compared with those treated during chronic HIV infection, has demonstrated a benefit for restoration of B cell functionality (21).

In conclusion, our study has shown that the B cell compartment is highly disturbed in chronically HIV-infected African women, an understudied group disproportionately affected by the HIV pandemic, as it is for Caucasian cohorts. Differences in methodology limit our ability to directly compare our results in African women to published studies in western cohorts, but we observed a substantial reduction in B cell activation and a relative normalization of skewed B cell subsets as observed in Caucasian cohorts, despite the commencement of ART in chronic HIV infection. Identifying the drivers of B cell activation and defective differentiation may provide a better understanding of the mechanisms associated with B cell dysfunction. This can potentially lead to the development of intervention strategies to improve B cell function during HIV infection, for both HIV-specific humoral immunity and responses to vaccines targeting other pathogens.

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Disclosures

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References

1. Appay, V., and D. Sauce. 2008. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J. Pathol.* 214: 231–241.
2. Moir, S., A. Malaspina, K. M. Ogwaro, E. T. Donoghue, C. W. Hallahan, L. A. Ehler, S. Liu, J. Adelsberger, R. Lapointe, P. Hwu, et al. 2001. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. *Proc. Natl. Acad. Sci. USA* 98: 10362–10367.
3. Moir, S., K. M. Ogwaro, A. Malaspina, J. Vasquez, E. T. Donoghue, C. W. Hallahan, S. Liu, L. A. Ehler, M. A. Planta, S. Kottilil, et al. 2003. Perturbations in B cell responsiveness to CD4+ T cell help in HIV-infected individuals. *Proc. Natl. Acad. Sci. USA* 100: 6057–6062.
4. Moir, S., J. Ho, A. Malaspina, W. Wang, A. C. DiPoto, M. A. O'Shea, G. Roby, S. Kottilil, J. Arthos, M. A. Proschan, et al. 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* 205: 1797–1805.
5. Malaspina, A., S. Moir, J. Ho, W. Wang, M. L. Howell, M. A. O'Shea, G. A. Roby, C. A. Rehm, J. M. Mican, T. W. Chun, and A. S. Fauci. 2006. Appearance of immature/transitional B cells in HIV-infected individuals with advanced disease: correlation with increased IL-7. *Proc. Natl. Acad. Sci. USA* 103: 2262–2267.
6. Malaspina, A., S. Moir, S. Kottilil, C. W. Hallahan, L. A. Ehler, S. Liu, M. A. Planta, T. W. Chun, and A. S. Fauci. 2003. Deleterious effect of HIV-1 plasma viremia on B cell costimulatory function. *J. Immunol.* 170: 5965–5972.
7. De Milito, A., A. Nilsson, K. Titanji, R. Thorstensson, E. Reizenstein, M. Narita, S. Grutzmeier, A. Sönnerborg, and F. Chiodi. 2004. Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. *Blood* 103: 2180–2186.
8. Kovacs, J. A., R. A. Lempicki, I. A. Sidorov, J. W. Adelsberger, B. Herpin, J. A. Metcalf, I. Sereti, M. A. Polis, R. T. Davey, J. Tavel, et al. 2001. Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *J. Exp. Med.* 194: 1731–1741.
9. Malaspina, A., S. Moir, S. M. Orsega, J. Vasquez, N. J. Miller, E. T. Donoghue, S. Kottilil, M. Gezmu, D. Follmann, G. M. Vodeiko, et al. 2005. Compromised B cell responses to influenza vaccination in HIV-infected individuals. *J. Infect. Dis.* 191: 1442–1450.
10. Titanji, K., A. De Milito, A. Cagigi, R. Thorstensson, S. Grützmeier, A. Atlas, B. Hejdeman, F. P. Kroon, L. Lopalco, A. Nilsson, and F. Chiodi. 2006. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. *Blood* 108: 1580–1587.
11. Hart, M., A. Steel, S. A. Clark, G. Moyle, M. Nelson, D. C. Henderson, R. Wilson, F. Gotch, B. Gazzard, and P. Kelleher. 2007. Loss of discrete memory B cell subsets is associated with impaired immunization responses in HIV-1

infection and may be a risk factor for invasive pneumococcal disease. *J. Immunol.* 178: 8212–8220.
12. French, N., C. F. Gilks, A. Mujugira, C. Fasching, J. O'Brien, and E. N. Janoff. 1998. Pneumococcal vaccination in HIV-1-infected adults in Uganda: humoral response and two vaccine failures. *AIDS* 12: 1683–1689.
13. Pasricha, N., U. Datta, Y. Chawla, S. Singh, S. K. Arora, A. Sud, R. W. Minz, B. Saikia, H. Singh, I. James, and S. Sehgal. 2006. Immune responses in patients with HIV infection after vaccination with recombinant Hepatitis B virus vaccine. *BMC Infect. Dis.* 6: 65.
14. Müller, F., P. Aukrust, I. Nordoy, and S. S. Froland. 1998. Possible role of interleukin-10 (IL-10) and CD40 ligand expression in the pathogenesis of hypergammaglobulinemia in human immunodeficiency virus infection: modulation of IL-10 and Ig production after intravenous Ig infusion. *Blood* 92: 3721–3729.
15. Swingler, S., J. Zhou, C. Swingler, A. Dauphin, T. Greenough, P. Jolicoeur, and M. Stevenson. 2008. Evidence for a pathogenic determinant in HIV-1 Nef involved in B cell dysfunction in HIV/AIDS. *Cell Host Microbe* 4: 63–76.
16. Lantto, R., A. Nasi, S. Sammicheli, S. Amu, V. Fievez, M. Moutschen, S. Pensieroso, B. Hejdeman, F. Chiodi, and B. Rethi. 2015. Increased extrafollicular expression of the B-cell stimulatory molecule CD70 in HIV-1-infected individuals. *AIDS* 29: 1757–1766.
17. Schnittman, S. M., H. C. Lane, S. E. Higgins, T. Folks, and A. S. Fauci. 1986. Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. *Science* 233: 1084–1086.
18. Swingler, S., B. Brichacek, J. M. Jacque, C. Ulich, J. Zhou, and M. Stevenson. 2003. HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-cell infection. *Nature* 424: 213–219.
19. D'Orsogna, L. J., R. G. Krueger, E. J. McKinnon, and M. A. French. 2007. Circulating memory B-cell subpopulations are affected differently by HIV infection and antiretroviral therapy. *AIDS* 21: 1747–1752.
20. Moir, S., A. Malaspina, J. Ho, W. Wang, A. C. Dipoto, M. A. O'Shea, G. Roby, J. M. Mican, S. Kottilil, T. W. Chun, et al. 2008. Normalization of B cell counts and subpopulations after antiretroviral therapy in chronic HIV disease. *J. Infect. Dis.* 197: 572–579.
21. Moir, S., C. M. Buckner, J. Ho, W. Wang, J. Chen, A. J. Waldner, J. G. Posada, L. Kardava, M. A. O'Shea, S. Kottilil, et al. 2010. B cells in early and chronic HIV infection: evidence for preservation of immune function associated with early initiation of antiretroviral therapy. *Blood* 116: 5571–5579.
22. Amu, S., G. Lavy-Shahaf, A. Cagigi, B. Hejdeman, S. Nozza, L. Lopalco, R. Mehr, and F. Chiodi. 2014. Frequency and phenotype of B cell subpopulations in young and aged HIV-1 infected patients receiving ART. *Retrovirology* 11: 76.
23. Pensieroso, S., L. Galli, S. Nozza, N. Ruffin, A. Castagna, G. Tambussi, B. Hejdeman, D. Misciagna, A. Riva, M. Malnati, et al. 2013. B-cell subset alterations and correlated factors in HIV-1 infection. *AIDS* 27: 1209–1217.
24. Rethi, B., S. Sammicheli, S. Amu, S. Pensieroso, B. Hejdeman, D. Schepis, P. H. Thang, and F. Chiodi. 2013. Concerted effect of lymphopenia, viraemia and T-cell activation on Fas expression of peripheral B cells in HIV-1-infected patients. *AIDS* 27: 155–162.
25. Abudulai, L. N., S. Fernandez, K. Corscadden, M. Hunter, L. A. Kirkham, J. J. Post, and M. A. French. 2016. Chronic HIV-1 infection induces B-cell dysfunction that is incompletely resolved by long-term antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* 71: 381–389.
26. van Grevenynghe, J., R. A. Cubas, A. Noto, S. Dafonseca, Z. He, Y. Peretz, A. Filali-mouhim, F. P. Dupuy, F. A. Procopio, N. Chomont, et al. 2011. Loss of memory B cells during chronic HIV infection is driven by Foxo3a- and TRAIL-mediated apoptosis. *J. Clin. Invest.* 121: 3877–3888.
27. Kroon, F. P., G. F. Rimmelzwaan, M. T. L. Roos, A. D. M. E. Osterhaus, D. Hamann, F. Miedema, and J. T. van Dissel. 1998. Restored humoral immune response to influenza vaccination in HIV-infected adults treated with highly active antiretroviral therapy. *AIDS* 12: F217–F223.
28. Whitaker, J. A., N. G. Rouphael, S. Edupuganti, L. Lai, and M. J. Mulligan. 2012. Strategies to increase responsiveness to hepatitis B vaccination in adults with HIV-1. *Lancet Infect. Dis.* 12: 966–976.
29. Béniguel, L., E. Bégaud, F. Cognasse, P. Gabrié, C. D. Mbolidi, O. Sabido, M. A. Marovich, C. DeFontaine, A. Frésard, F. Lucht, et al. 2004. Identification of germinal center B cells in blood from HIV-infected drug-naive individuals in Central Africa. *Clin. Dev. Immunol.* 11: 23–27.
30. Longwe, H., S. Gordon, R. Malamba, and N. French. 2010. Characterising B cell numbers and memory B cells in HIV infected and uninfected Malawian adults. *BMC Infect. Dis.* 10: 280.
31. Eggena, M. P., B. Barugahare, M. Okello, S. Mutyala, N. Jones, Y. Ma, C. Kityo, P. Mugyenyi, and H. Cao. 2005. T cell activation in HIV-seropositive Ugandans: differential associations with viral load, CD4+ T cell depletion, and coinfection. *J. Infect. Dis.* 191: 694–701.
32. Roetynck, S., A. Olotu, J. Simam, K. Marsh, B. Stockinger, B. Urban, and J. Langhorne. 2013. Phenotypic and functional profiling of CD4 T cell compartment in distinct populations of healthy adults with different antigenic exposure. *PLoS One* 8: e55195.
33. Monath, T. P., R. Nichols, W. T. Archambault, L. Moore, R. Marchesani, J. Tian, R. E. Shope, N. Thomas, R. Schrader, D. Furby, and P. Bedford. 2002. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial. *Am. J. Trop. Med. Hyg.* 66: 533–541.
34. Muyanja, E., A. Ssemaganda, P. Ngauv, R. Cubas, H. Perrin, D. Srinivasan, G. Canderan, B. Lawson, J. Kopycinski, A. S. Graham, et al. 2014. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. [Published erratum appears in 2014 *J. Clin. Invest.* 124: 4669.] *J. Clin. Invest.* 124: 3147–3158.
35. Meier, A., J. J. Chang, E. S. Chan, R. B. Pollard, H. K. Sidhu, S. Kulkarni, T. F. Wen, R. J. Lindsay, L. Orellana, D. Mildvan, et al. 2009. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat. Med.* 15: 955–959.
36. Hagen, S., and M. Altfeld. 2016. The X awakens: multifactorial ramifications of sex-specific differences in HIV-1 infection. *J. Virus Erad.* 2: 78–81.
37. van Loggerenberg, F., K. Mlisana, C. Williamson, S. C. Auld, L. Morris, C. M. Gray, Q. Abdool Karim, A. Grobler, N. Barnabas, I. Iriogbe, and S. S. Abdool Karim, CAPRISA 002 Acute Infection Study Team. 2008. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. *PLoS One* 3: e1954.
38. Mlisana, K., L. Werner, N. J. Garrett, L. R. McKinnon, F. van Loggerenberg, J. A. S. Passmore, C. M. Gray, L. Morris, C. Williamson, and S. S. Abdool Karim, Centre for the AIDS Programme of Research in South Africa (CAPRISA) 002 Study Team. 2014. Rapid disease progression in HIV-1 subtype C-infected South African women. *Clin. Infect. Dis.* 59: 1322–1331.
39. Abdool Karim, Q., S. S. Abdool Karim, J. A. Frohlich, A. C. Grobler, C. Baxter, L. E. Mansoor, A. B. Kharsany, S. Sibeko, K. P. Mlisana, Z. Omar, et al; CAPRISA 004 Trial Group. 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 329: 1168–1174.
40. Moir, S., and A. S. Fauci. 2009. B cells in HIV infection and disease. *Nat. Rev. Immunol.* 9: 235–245.
41. Deeks, S. G., C. M. R. Kitchen, L. Liu, H. Guo, R. Gascon, A. B. Narváez, P. Hunt, J. N. Martin, J. O. Kahn, J. Levy, et al. 2004. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 104: 942–947.
42. Hunt, P. W., J. N. Martin, E. Sinclair, B. Bredt, E. Hagos, H. Lampiris, and S. G. Deeks. 2003. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J. Infect. Dis.* 187: 1534–1543.
43. Brenchley, J. M., D. A. Price, T. W. Schacker, T. E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, et al. 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12: 1365–1371.
44. Moir, S., and A. S. Fauci. 2013. Insights into B cells and HIV-specific B-cell responses in HIV-infected individuals. *Immunol. Rev.* 254: 207–224.
45. Ho, J., S. Moir, A. Malaspina, M. L. Howell, W. Wang, A. C. DiPoto, M. A. O'Shea, G. A. Roby, R. Kwan, J. M. Mican, et al. 2006. Two overrepresented B cell populations in HIV-infected individuals undergo apoptosis by different mechanisms. *Proc. Natl. Acad. Sci. USA* 103: 19436–19441.
46. Titanji, K., F. Chiodi, R. Bellocco, D. Schepis, L. Osorio, C. Tassandin, G. Tambussi, S. Grutzmeier, L. Lopalco, and A. De Milito. 2005. Primary HIV-1 infection sets the stage for important B lymphocyte dysfunctions. *AIDS* 19: 1947–1955.
47. Moir, S., A. Malaspina, Y. Li, T. W. Chun, T. Lowe, J. Adelsberger, M. Baseler, L. A. Ehler, S. Liu, R. T. Davey, Jr., et al. 2000. B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. *J. Exp. Med.* 192: 637–646.
48. Qiao, X., B. He, A. Chiu, D. M. Knowles, A. Chadburn, and A. Cerutti. 2006. Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. *Nat. Immunol.* 7: 302–310.
49. Xu, W., P. A. Santini, J. S. Sullivan, B. He, M. Shan, S. C. Ball, W. B. Dyer, T. J. Ketas, A. Chadburn, L. Cohen-Gould, et al. 2009. HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells via long-range intercellular conduits. *Nat. Immunol.* 10: 1008–1017.
50. Ruffin, N., P. H. Thang, B. Rethi, A. Nilsson, and F. Chiodi. 2012. The impact of inflammation and immune activation on B cell differentiation during HIV-1 infection. *Front. Immunol.* 2: 90.
51. Fontaine, J., J. Chagnon-Choquet, H. S. Valcke, J. Poudrier, and M. Roger, Montreal Primary HIV Infection and Long-Term Non-Progressor Study Groups. 2011. High expression levels of B lymphocyte stimulator (BLyS) by dendritic cells correlate with HIV-related B-cell disease progression in humans. *Blood* 117: 145–155.
52. Poudrier, J., C. Soulas, J. Chagnon-Choquet, T. Burdo, P. Autissier, K. Oskar, K. C. Williams, and M. Roger. 2015. High expression levels of BLyS/BAFF by blood dendritic cells and granulocytes are associated with B-cell dysregulation in SIV-infected rhesus macaques. *PLoS One* 10: e0131513.
53. Gomez, A. M., M. Ouellet, A. Deshiere, Y. Breton, and M. J. Tremblay. 2016. HIV-1-mediated BAFF secretion in macrophages does not require endosomal TLRs, type-I IFN, and Nef, but depends on the cellular phenotype status. *J. Immunol.* 196: 3806–3817.
54. Jiang, W. 2012. Microbial translocation and B cell dysfunction in human immunodeficiency virus disease. *Am. J. Immunol.* 8: 44–51.
55. Hornung, V., S. Rothenfusser, S. Britsch, A. Krug, B. Jahrsdörfer, T. Giese, S. Endres, and G. Hartmann. 2002. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J. Immunol.* 168: 4531–4537.
56. Jiang, W., M. M. Lederman, P. Hunt, S. F. Sieg, K. Haley, B. Rodriguez, A. Landay, J. Martin, E. Sinclair, A. I. Asher, et al. 2009. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J. Infect. Dis.* 199: 1177–1185.
57. He, B., X. Qiao, and A. Cerutti. 2004. CpG DNA induces IgG class switch DNA recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-10. *J. Immunol.* 173: 4479–4491.

58. Jiang, W., M. M. Lederman, C. V. Harding, B. Rodriguez, R. J. Mohner, and S. F. Sieg. 2007. TLR9 stimulation drives naïve B cells to proliferate and to attain enhanced antigen presenting function. *Eur. J. Immunol.* 37: 2205–2213.
59. Noronha, A. M., Y. Liang, J. T. Hetzel, H. Hasturk, A. Kantarci, A. Stucchi, Y. Zhang, B. S. Nikolajczyk, F. A. Farraye, and L. M. Ganley-Leal. 2009. Hyperactivated B cells in human inflammatory bowel disease. *J. Leukoc. Biol.* 86: 1007–1016.
60. d'Ettorre, G., S. Baroncelli, L. Micci, G. Ceccarelli, M. Andreotti, P. Sharma, G. Fanello, F. Fiocca, E. N. Cavallari, N. Giustini, et al. 2014. Reconstitution of intestinal CD4 and Th17 T cells in antiretroviral therapy suppressed HIV-infected subjects: implication for residual immune activation from the results of a clinical trial. *PLoS One* 9: e109791.
61. Hattab, S., A. Guihot, M. Guiguet, S. Fourati, G. Carcelain, F. Caby, A. G. Marcelin, B. Autran, D. Costagliola, and C. Katlama. 2014. Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infect. Dis.* 14: 122.
62. Rudy, B. J., B. G. Kapogiannis, C. Worrell, K. Squires, J. Bethel, S. Li, C. M. Wilson, A. Agwu, P. Emmanuel, G. Price, et al; Adolescent Trials Network for HIVAIDS Interventions. 2015. Immune reconstitution but persistent activation after 48 weeks of antiretroviral therapy in youth with pre-therapy CD4 >350 in ATN 061. *J. Acquir. Immune Defic. Syndr.* 69: 52–60.
63. Haas, A., K. Zimmermann, and A. Oxenius. 2011. Antigen-dependent and -independent mechanisms of T and B cell hyperactivation during chronic HIV-1 infection. *J. Virol.* 85: 12102–12113.
64. Amu, S., V. Fievez, S. Nozza, L. Lopalco, and F. Chiodi. 2016. Dysfunctions in the migratory phenotype and properties of circulating immature transitional B cells during HIV-1 infection. *AIDS* 30: 2169–2177.
65. Pensieroso, S., A. Cagigi, P. Palma, A. Nilsson, C. Capponi, E. Freda, S. Bernardi, R. Thorstensson, F. Chiodi, and P. Rossi. 2009. Timing of HAART defines the integrity of memory B cells and the longevity of humoral responses in HIV-1 vertically-infected children. *Proc. Natl. Acad. Sci. USA* 106: 7939–7944.
66. Van Epps, P., R. M. Matining, K. Tassiopoulos, D. D. Anthony, A. Landay, R. C. Kalayjian, and D. H. Canaday. 2014. Older age is associated with peripheral blood expansion of naïve B cells in HIV-infected subjects on antiretroviral therapy. *PLoS One* 9: e107064.
67. Levesque, M. C., M. A. Moody, K. K. Hwang, D. J. Marshall, J. F. Whitesides, J. D. Amos, T. C. Gurley, S. Allgood, B. B. Haynes, N. A. Vandergrift, et al. 2009. Polyclonal B cell differentiation and loss of gastrointestinal tract germinal centers in the earliest stages of HIV-1 infection. *PLoS Med.* 6: e1000107.
68. Klatt, N. R., N. T. Funderburg, and J. M. Brenchley. 2013. Microbial translocation, immune activation, and HIV disease. *Trends Microbiol.* 21: 6–13.
69. Ghislain, M., J. P. Bastard, L. Meyer, J. Capeau, S. Fellahi, L. Gérard, T. May, A. Simon, C. Vigouroux, and C. Goujard, ANRS-COPANA Cohort Study Group. 2015. Late antiretroviral therapy (ART) initiation is associated with long-term persistence of systemic inflammation and metabolic abnormalities. *PLoS One* 10: e0144317.
70. Fogli, M., C. Torti, F. Malacarne, S. Fiorentini, M. Albani, I. Izzo, C. Giagulli, F. Maggi, G. Carosi, and A. Caruso. 2012. Emergence of exhausted B cells in asymptomatic HIV-1-infected patients naïve for HAART is related to reduced immune surveillance. *Clin. Dev. Immunol.* 2012: 829584.
71. Wheatley, A. K., A. B. Kristensen, W. N. Lay, and S. J. Kent. 2016. HIV-dependent depletion of influenza-specific memory B cells impacts B cell responsiveness to seasonal influenza immunisation. *Sci. Rep.* 6: 26478.