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Immune perturbations in HIV–1-infected individuals who make broadly reactive neutralizing antibodies

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Abstract

Induction of broadly neutralizing antibodies (bnAbs) is a goal of HIV-1 vaccine development. BnAbs occur in some HIV-1-infected individuals and frequently have characteristics of

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Author Contributions

BFH conceived and designed the study, oversaw and directed autoreactivity and plasma protein binding assays, evaluated all data, wrote the paper; IPP developed methods for and performed flow analysis of $CD4^+$ T cell subsets, contributed to data analysis; NAV performed all statistical analysis in the paper, edited the paper; CC developed methods for and performed flow analysis of $CD4^+$ T cell subsets; KEL performed autoreactivity and plasma protein binding assays; RP performed autoreactivity and plasma protein binding assays; KAS managed programmatic aspects of the study including sample annotation and cohort analysis; MSC led the team that acquired the samples in Cohort A; HXL provided recombinant protein reagents for plasma binding assays; FG performed clade typing of HIV-1; AJM designed experiments and evaluated $CD4^+$ T cell flow data; DCM performed neutralization assays; LV designed experiments and evaluated data; GK designed experiments, evaluated data, and edited the paper; JH screened, recruited, and performed neutralization assays on Cohort B; PRS performed the exome sequencing study and association analysis; MC provided samples for Cohort B, wrote and edited the paper; PB designed and directed the $CD4^+$ T cell subset analyses, evaluated all data, wrote and edited the paper; and MAM designed the study, wrote and edited the paper, and analyzed all data.

autoantibodies. Here we have studied cohorts of HIV-1-infected individuals that made bnAbs and compared them to those who did not do so, and determined immune traits associated with the ability to produce bnAbs. HIV-1-infected individuals with bnAbs had a higher frequency of blood autoantibodies, a lower frequency of regulatory $CD4^+$ T cells, a higher frequency of circulating memory T follicular helper $CD4^+$ cells and a higher T regulatory cell level of programmed cell death-1 expression compared to HIV-1-infected individuals without bnAbs. Thus, induction of HIV-1 bnAbs may require vaccination regimens that transiently mimic immunologic perturbations in HIV-1-infected individuals.

Introduction

Development of a HIV-1 vaccine is the cornerstone of a comprehensive global HIV-1 preventive strategy (1). A critical component of a successful strategy is design of immunogens to induce broadly-reactive neutralizing antibodies (bnAbs) (2, 3). However, to date, no candidate HIV-1 vaccines have induced plasma bnAb activity (4–6). Only rarely do HIV-1-infected individuals make high levels of bnAbs, but over 2–4 years of infection, ~50% of infected individuals develop cross-reactive antibodies that neutralize ~50% of difficult-to-neutralize (tier 2) HIV-1 isolates (7–11).

Clues to the dearth of vaccine-induced bnAbs come from analysis of the physical characteristics of bnAbs (2, 12–14). All bnAbs isolated to date either have very high frequencies of somatic mutations, long third heavy chain complementarity determining regions, and/or autoreactivity—traits of B-cell antigen receptors that are negatively regulated by immune tolerance mechanisms (13, 15–26). One hypothesis is that some, or all bnAb development may be controlled by one or more immune tolerance mechanisms (2, 12, 13, 16).

Approximately 50% of HIV-1-infected individuals will produce autoantibodies during untreated HIV-1 infection (27–33). Here we have profiled the immune perturbations associated with HIV-1 infection and bnAb induction. We studied a cohort of 239 chronically HIV-1-infected individuals for serum ability to broadly neutralize HIV-1 strains, and selected 51 individuals with the highest blood bnAb activity versus 51 matched individuals with low neutralizing activity. We tested for the coincident presence of plasma autoantibodies, and for peripheral blood $CD4^+$ T cell subset frequencies in the HIV-1-infected individuals that had made bnAbs, in those that had not made bnAbs, and in HIV-1-seronegative controls (including those with and without autoantibodies). We quantified total $CD4^+$ T cells and assessed the circulating frequency of resting memory T follicular helper cells (mTfh cells), a population defined as the PD-1^+ $CXCR3^-$ subset of $CXCR5^+$ $CD4^+$ T cells (34). We also determined the frequency of $CD25^+$ $Foxp3^+$ regulatory $CD4^+$ T (Treg) cells — a population of cells that have been shown to suppress the development of autoantibody responses [reviewed in (35)] — as well as the proportion of circulating $CD4^+$ T follicular-phenotype cells (ie, $CD4^+$ $CXCR5^+$ T cells) composed of $CD25^+$ $Foxp3^+$ $CD4^+$ T follicular regulatory (Tfr) cells, the subset of $CD4^+$ Treg cells that mediates control of B cell responses within germinal centers (36–42), and measured PD-1 expression on both Treg and Tfr cells.

Autoantibodies in HIV-1-infected individuals with high versus low HIV-1 neutralizing activity in plasma

Cohort A consisted of 239 HIV-1-infected individuals of whom 214 (90%) were African (Table S1 online). Based on serum neutralization data, we selected 51 individuals who had the highest level of bnAbs and matched them with 51 individuals who did not have bnAbs (Figure 1A; Figures S1–2 and Tables S1–9 online). Thirty-three of 51 (65% of) HIV-1-infected individuals with bnAb activity had positive plasma reactivity in one or more autoantibody assays (Figure 1C). In contrast, only 16 of 51 (31%) in the non-bnAb HIV-1-infected control group had plasma autoantibodies ($\chi^2 = 11.4$, $p = 0.0008$; Figure 1C). Poisson regression results also showed the bnAb individuals to have a higher number of positive autoantibody tests than the non-bnAb HIV-1-infected control group ($\chi^2 = 14.7$, $p = 0.0001$; Figure 1E). Cohort A was infected primarily with clade C viruses (A.bnAb 33/51, 65%; A.Control 37/51, 73%; $p = 0.52$, Fisher's exact test; Table S9 online), but we found that clade of infecting virus had no effect on either the presence of neutralization breadth or the presence of autoantibodies (Tables S10–12 online).

To determine the effect of geographic region of HIV-1 individual origin on autoantibody frequency in bnAb individuals, we studied a second confirmatory group (Cohort B; Tables S13–16 online) of HIV-1 infected individuals from the US comprised of subjects who produced bnAbs and those who had lower levels of plasma neutralizing antibodies (Figure 1B). Confirming the data in Cohort A, we found that 22 of 24 (92%) HIV-1-infected individuals in Cohort B with bnAb activity had at least one positive test, while only 12 of 21 (57%) Cohort B low level neutralizing antibody individuals had plasma autoantibodies ($\chi^2 = 7.2$, $p = 0.007$; Figure 1D). However, due to the lower number of subjects in Cohort B, Poisson regression analysis was unable to show a difference in the total number of positive autoantibody tests in Cohort B bnAb individuals than the non-bnAb controls ($\chi^2 = 1.8$, $p = 0.2$; Figure 1F). Moreover, the Cohort B HIV-1-infected non-bnAb control group was different from the non-bnAb control group of Cohort A. The US Cohort B control group had a greater level of neutralizing antibodies than the primarily African Cohort A control group (B Control total isolate tested geometric mean titer [GMT] = 32.7, A Control GMT = 12.2, $p < 0.0001$, *t*-test; Tables S4, S14 online).

Since HIV-1 infection is associated with polyclonal B cell activation (43), we next asked if overall levels of antibody production to HIV-1 and non-HIV-1 antigens were similar between the bnAb and control groups of both cohorts. Thus, we assayed for plasma antibody binding to HIV-1 Env gp120 and gp41, as well as to trivalent inactivated influenza vaccine (TIV2008). Binding to HIV-1 Env gp120 ($p < 0.0001$, *t*-test) and gp41 ($p < 0.0001$, *t*-test) proteins were elevated in the Cohort A bnAb group compared to the Cohort A control group (Figure 2A, 2B; Table S17 online), while plasma antibodies to influenza were similar between the Cohort A control group and the bnAb group ($p = 0.19$, *t*-test; Figure 2C). In both Cohort B bnAb and control groups, antibodies to gp120, gp41 and TIV2008 were not statistically different ($p = 0.35$, 0.46, 0.27, respectively, *t*-test; Figure 2D–F; Table S17 online). Thus, the lack of increased antibodies to influenza in these cohorts suggests that the higher frequency of autoantibodies in HIV-1-infected individuals with bnAbs was not due to a general increase in antibody induction.

CD4+ T cell subsets in Cohort A bnAb group versus Cohort A control groups

CD25+ Foxp3+ CD4+ Treg cells play an important role in prevention of autoimmunity, and loss or impairment of Treg function leads to autoantibody induction (35, 44, 45). Conversely, increased frequencies of CD4+ T follicular helper (Tfh) cells, which play a crucial role in the germinal center B-cell response, are often associated with autoantibody production (46–48). Thus, we determined the frequency of CD4+ Tfh and Treg cells in the Cohort A bnAb and non-bnAb groups from whom PBMC samples were available, and matched HIV-1-seronegative controls. The A.bnAb group had a higher mean viral load than the non-bnAb group (Table S7 online), although we found that the presence of autoantibodies was independent of viral load (Tables S19–20 online). Total CD4+ T cells in both groups of HIV-1-infected individuals were reduced compared to HIV-1-seronegative controls, and they were significantly lower in the A.bnAb group than in the A.Control group ($p = 0.0001$, *t*-test; Figure 3A; Table S18 online). Analysis of the circulating frequency of resting memory T follicular helper (mTfh) CD4+ T cells, defined as the PD-1+ CXCR3− subset of CXCR5+ CD4+ T cells (34), revealed that mTfh were present at significantly higher frequencies in the A.bnAb group than in the A.Control group or HIV-1-seronegative controls ($p < 0.0001$ and $p < 0.0001$, respectively, *t*-test; Figure 3B; Table S18 online). The frequency of CD25+ Foxp3+ CD4+ Treg cells within lymphocytes was also significantly lower in the A.bnAb group compared to the A.Control group ($p = 0.004$, *t*-test; Figure 3C; Table S18 online), although the frequency of Treg cells within CD4+ T cells did not differ significantly between groups (not shown).

The inhibitory receptor programmed cell death-1 (PD-1) has been associated with CD4+ and CD8+ T cell activation and/or dysfunction in HIV-1 infection (reviewed in (49)) and with the development of autoimmunity (reviewed in (50)). Thus, we measured the level of PD-1 on circulating CD25+ Foxp3+ CD4+ Treg cells, and found that in the A.bnAb group Treg cells expressed significantly higher levels of the inhibitory receptor PD-1 than Tregs in A.Control individuals ($p < 0.0001$, *t*-test) or in HIV-1-seronegative controls ($p < 0.0001$, *t*-test; Figure 3D; Table S18 online). Importantly, the differences in mTfh and Treg frequencies and PD-1 expression on Tregs in the A.bnAb and A.Control groups were independent of their differing viral loads (Tables S21–22 online).

Recent studies have identified a subpopulation of CD25+ Foxp3+ CD4+ Treg cells that shares some of the phenotypic characteristics of CD4+ T follicular helper (Tfh) cells including expression of Bcl6, CXCR5, PD-1 and ICOS; these CD4+ T follicular regulatory (Tfr) cells can home into germinal centers, and regulate the germinal center response by limiting Tfh numbers and function and via direct effects on B cells (35, 37–42, 51, 52). Germinal center B cell responses are thought to be determined by the relative proportions of Tfr and Tfh cells, rather than the number of Tfr cells (41). Memory Tfr populations circulate in peripheral blood (41). We therefore measured the proportion of CD25+ Foxp3+ regulatory cells in the circulating CD4+ T follicular-phenotype cell population, defined as CXCR5+ CD4+ T cells. In both Cohort A groups of HIV-1-infected individuals, the proportion of Tfr cells within the circulating CXCR5+ CD4+ T cell population (Figure 3E; Table S18 online) and the Tfr/Tfh ratio (ie, ratio of CD25+ Foxp3+ to non-CD25+ Foxp3+ cells in the circulating CXCR5+ CD4+ T cell population) (Figure 3F; Table S18 online) did not differ

significantly in the A.bnAb and the A.Control groups, although the level of PD-1 expression on Tfr cells was significantly higher in the former group ($p = 0.004$, *t*-test; Figure 3G; Table S18 online). Given the current lack of consensus about the phenotypic definition of circulating $CD4^+$ Tfr cells in humans (41, 53–55), we also considered two other definitions for this population ($CD25^+$ $Foxp3^+$ cells within $CXCR3^-$ PD-1^+ $CXCR5^+$ $CD4^+$ T cells, and $CD25^+$ $Foxp3^+$ cells within $ICOS^+$ PD-1^+ $CXCR5^+$ $CD4^+$ T cells). Similar results were obtained regardless of the definition used, with no significant difference being observed in circulating Tfr frequencies or the Tfr/Tfh ratio in the A.bnAb and the A.Control groups, and PD-1 on Tfr being significantly higher or showing a trend towards higher expression in the A.bnAb group (Figure S6; Table S23 online). Interestingly, when we divided the Cohort A subjects into groups on the basis of generation of autoantibodies rather than bnAbs, we found that autoantibody-positive subjects exhibited similar alterations in T cell subsets, including expression of higher levels of PD-1 on $CD4^+$ Treg and Tfr populations, to that seen between the A.bnAb and A.Control groups, although this difference was less pronounced and did not reach statistical significance (data not shown).

The higher levels of PD-1 expression on $CD4^+$ Treg and Tfr cells in the A.bnAb group raised the hypothesis that regulatory $CD4^+$ T cell populations in the A.bnAb group are more activated and may be more dysfunctional than those in the A.Control group. We thus compared the expression of HLA-DR (another marker associated with $CD4^+$ T cell activation) and CTLA-4 and LAG-3 (both of which are markers of $CD4^+$ T cell exhaustion (56), but are also involved in $CD4^+$ Treg cell effector function (57, 58)) on $CD4^+$ Treg cells in the Cohort A bnAb and non-bnAb groups and matched HIV-1-seronegative controls. The percentage of $CD4^+$ Treg cells expressing HLA-DR was significantly higher in the A.bnAb than the A.control group ($p = 0.0003$, *t*-test; Figure 4A; Table S24 online), and the level of PD-1 expression on Tregs was also significantly higher in the former group (not shown). Similar observations were also made for CTLA-4 ($p = 0.01$, *t*-test; Figure 4B; Table S24 online); and although there was no difference between groups in the percentage of $CD4^+$ Treg cells expressing LAG-3 (Figure 4C; Table S24 online), the level of LAG-3 expression on $CD4^+$ Tregs was again significantly higher in the A.bnAb than the A.control group (not shown). Notably, HLA-DR, CTLA-4 and LAG-3 were all expressed on a significantly higher proportion of PD-1^{high} $CD4^+$ Treg cells than PD-1^{low} or PD-$1^{negative}$ $CD4^+$ Tregs in both HIV-1-infected (data for the A.bnAb group shown in Figure 4D–F; Table S25 online) and HIV-1 seronegative control subjects (Figure 4G–I; Table S26 online). To explore the functional capacity of the PD-1^{high} subset of $CD4^+$ Treg cells, $CD25^{high}$ $CD127^{low}$ $CD4^+$ Treg cells from HIV-1-seronegative donors were sorted into PD-1^{high}, PD-1^{low} and PD-$1^{negative}$ subpopulations and their ability to suppress the proliferation of conventional ($CD25^{low}$ $CD127^{high}$) $CD4^+$ T cells assessed. Whereas PD-1^{low} and PD-$1^{negative}$ $CD4^+$ Treg subpopulations mediated significant suppression of conventional $CD4^+$ T cell proliferation ($p = 0.01$, sign test; Figure 4J, Table S27 online), this was not the case for PD-1^{high} $CD4^+$ Treg cells—the PD-1^{high} $CD4^+$ Treg subpopulation from some donors had a highly impaired suppressive capacity (Figure 4J). Together, these results suggested that high PD-1 expression on regulatory $CD4^+$ T cells, as observed in the A.bnAb group, is indicative of activation and development of an impaired functional capacity.

The higher frequency of blood autoantibodies, higher frequency of circulating $CD4^+$ mTfh cells, lower frequency of $CD4^+$ Treg cells, and higher levels of PD-1 expression on $CD4^+$ Treg and Tfr cells observed in the A.bnAb as compared to the A.control group may have been present prior to HIV-1 infection, and/or may have developed or been accentuated during the course of infection. The Cohort A subjects had not been sampled prior to or during the early stages of HIV-1 infection and we were unable to address the sequence of events preceding bnAb development during the course of infection. However, to gain insight into whether some healthy individuals have a pre-existing immunological profile that could potentially predispose them to bnAb induction following HIV acquisition, we analyzed $CD4^+$ T cell subsets in healthy HIV-1-seronegative individuals with and without plasma autoantibodies. None of the 48 HIV-1-seronegative control individuals studied in parallel to the HIV-1-infected groups had plasma autoantibodies (data not shown). However, when we screened for the presence of autoantibodies in 118 predominantly African HIV-1-seronegative individuals, we identified 12 individuals who did have plasma autoantibodies. Analysis of total and regulatory $CD4^+$ T cell populations in these individuals and a control group of 23 age, sex, and location-matched HIV-1-seronegative individuals without plasma autoantibodies revealed that there was no difference between groups in the frequency of total $CD4^+$ T cells within lymphocytes or circulating frequency of mTfh cells (Figures 5A and 5B; Table S28 online); but that the frequency of $CD25^+$ $Foxp3^+$ $CD4^+$ Treg cells in the HIV-1-uninfected individuals with autoantibodies was lower than that in those without autoantibodies, although not statistically significant ($p = 0.06$, *t*-test; Figure 5C; Table S28 online). The level of PD-1 expression on $CD4^+$ Treg cells in some of the HIV-1-uninfected individuals with autoantibodies was also higher than that in those without autoantibodies, although there was not a significant difference between groups in PD-1 expression on $CD4^+$ Treg cells overall ($p = 0.63$, *t*-test; Figure 5D; Table S28 online).

Since HLA allotypes have been associated with the development of autoimmune disease (59), we performed HLA typing on all individuals in Cohort A and found no significant differences in distribution between bnAb versus control groups for HLA class I (Tables S29–30 online) or class II allotypes (Tables S31–32 online) (Cochran-Mantel-Haenszel tests).

Finally, to look for evidence of genes that predisposed HIV-1-infected individuals to make bnAbs, we performed full exome sequencing on the 51 bnAb individuals and on the 51 control HIV-1-infected individuals without bnAbs. We found no genome-wide significant mutations in either group, although we did identify 20 single nucleotide variants or small indels in the association study of HIV-1 broad neutralization before genome-wide statistical correction (Table S33 online). To focus the analysis, we compared only those bnAb individuals that also expressed plasma autoantibody reactivity to individuals in the non-bnAb group that showed no autoantibody activity. Again no significant genome-wide associations were found, however a number of candidate genes were identified that are known to be relevant to control of the immune system (Table S34 online).

Discussion

A major conundrum in the HIV-1 vaccine development field is why 50% of HIV-1-infected individuals make bnAbs after years of infection, but vaccination of uninfected individuals

with antigenic HIV-1 envelopes has, as yet, not induced bnAbs. While structural integrity of the native envelope immunogen is a critical component for induction of bnAbs (3), multiple envelope trimer immunization studies have yet to induce bnAbs (60–66). Here we have defined the profile of immune perturbations found in those HIV-1-infected individuals with plasma bnAbs. HIV-1-infected individuals that make cross-reactive neutralizing antibodies have a higher viral load, lower total $CD4^+$ T cells, higher frequency of blood autoantibodies, higher levels of circulating mTfh, a lower frequency of Treg cells and higher levels of PD-1 on Treg and Tfr cells compared to a group of HIV-1-infected individuals with no or low bnAb levels.

Early in the AIDS epidemic before anti-retroviral treatment, it was noted that HIV-1 infection induced host immunoregulatory abnormalities leading to plasma autoantibody production (27, 29, 31, 43), and a high incidence of autoantibody seropositivity (27–30, 32, 33). In an earlier pilot study of 16 HIV-1-infected individuals, we found anti-cardiolipin antibodies were frequently present in those with plasma neutralization breadth but no significant elevation of other autoantibodies (67). The findings in our current study provide evidence for the hypothesis that one reason that bnAbs are induced in some HIV-1-infected individuals is that HIV-1 infection perturbs their immune system by loss, activation, and/or exhaustion of $CD25^+$ $Foxp3^+$ regulatory $CD4^+$ T cell populations in the setting of elevated $CD4^+$ Tfh cells, thus facilitating the production of bnAbs. It would be of interest to prospectively recruit a new group of acutely HIV-1-infected individuals to determine the mechanism and timing of these events rather than use retrospective samples. However, with evidence supporting the early initiation of antiretroviral therapy at the time of diagnosis (68), it is no longer ethical to perform natural history studies of HIV-1 infection without offering antiretroviral therapy.

In HIV-1 infection, $CD4^+$ T cells, including $CD25^+$ $Foxp3^+$ $CD4^+$ Treg cells, are lost as a consequence of infection with HIV-1 and bystander apoptosis (69, 70). The Cohort A HIV-1-infected individuals producing bnAbs had higher viral loads than those subjects not producing bnAbs, and higher viral loads have also been associated with bnAb production in other HIV-1-infected cohorts (reviewed in (71)). However, although the high viral loads in bnAb individuals may have been among the factors contributing to the greater depletion of both total $CD4^+$ T cells and $CD4^+$ Tregs in the subjects producing bnAbs in our study, all the differences observed in $CD4^+$ T cell subsets in the groups of subjects producing or not producing bnAbs were independent of viral load, indicating that they were primarily driven by other factors.

Tfh cell differentiation is a multistep process regulated by numerous signals; however, cytokines play an important role in regulation of early Tfh differentiation, with signaling via IL-6 promoting and via IL-2 inhibiting Tfh differentiation (72). Tfh cells are thus expanded in a number of chronic viral infections including HIV-1 where IL-6 is induced and IL-2 is limited (34, 73–77). In a previous study of an HIV-1-infected cohort, chronically-infected individuals generating bnAbs were found to have higher circulating frequencies of mTfh than matched individuals not generating bnAbs (34). Locci et al. further showed that mTfh frequencies during early HIV-1 infection were higher in subjects who subsequently

developed bnAbs; likewise Cohen et al. reported an association between early preservation of PD1$^+$ CXCR5$^+$ CD4$^+$ Tfh cells and bnAb development (78).

A recent study proposed that the expansion of germinal centre Tfh cells during SIV infection may be facilitated by a decline in the Tfr/Tfh ratio (79). However others have reported an increase in the frequency of Tfr in lymph nodes and spleen during SIV/HIV infection, which suggests that the lymph node Tfr/Tfh ratio may not be perturbed (80, 81). Here, we studied circulating CD4$^+$ Treg and Tfr cell subsets. We found that compared to subjects without bnAbs, subjects with bnAbs had a lower frequency of CD4$^+$ Treg cells within lymphocytes; but although CD4$^+$ Tfr largely differentiate from Tregs (37–39, 82), the circulating frequency of Tfr did not differ significantly between groups. Notably, PD-1 was expressed at significantly higher levels on both CD4$^+$ Treg and CD4$^+$ Tfr cells in subjects with bnAbs. Elevated PD-1 expression in the bnAb group likely reflects higher levels of immune activation in these subjects, consistent with which CD4$^+$ Treg cells also expressed higher levels of the activation marker HLA-DR. PD-1 is an inhibitory receptor, ligation of which has been shown to inhibit Tfr function in mice (41). PD-L1 expression on lymph node B cells is increased during HIV-1 infection (77), hence the function of Tfr in subjects producing bnAbs may be inhibited as a consequence of their elevated expression of PD-1. PD-1 expression can also reflect T cell exhaustion as a consequence of sustained activation, and CD4$^+$ Treg cells from subjects producing bnAbs expressed elevated levels of CTLA-4 and LAG-3, markers indicative of CD4$^+$ T cell exhaustion (56). Despite the fact that CTLA-4 and LAG-3 are also involved in Treg and Tfr function (40, 57, 58), we found that PD-1^{high} CD4$^+$ Treg cells exhibited an impaired ability to suppress the proliferation of conventional CD4$^+$ T cells in vitro, supporting the hypothesis that elevated PD-1 expression on regulatory CD4$^+$ T cell subsets may reflect cellular exhaustion and an impaired suppressive capacity.

Our data suggest that by the time of chronic HIV-1 infection, the majority of viremic individuals develop alterations in the CD4$^+$ T cell subsets that mediate control of germinal center B-cell responses. High viral loads, immune activation, dysregulation of cytokine production and alterations in lymphoid tissue microenvironments may drive the development of particularly profound abnormalities in regulatory CD4$^+$ T cell subsets in some subjects, creating an environment permissive to generation of both autoantibodies and bnAbs that then emerge in a subset of these individuals. However, since some HIV-1-infected individuals with bnAbs did not have autoantibodies, the propensity to make autoantibodies may be a surrogate marker for an as yet undiscovered perturbation induced by HIV-1 that leads to bnAb induction. Moreover, we cannot rule out that those HIV-1-infected individuals without plasma bnAbs but with autoantibodies may eventually go on to develop bnAbs, but had not done so at the time of study.

Some individuals may also be predisposed to generate bnAbs following infection with HIV-1 due to pre-existing abnormalities in host tolerance controls. Increased Tfh frequencies and loss or functional impairment of regulatory CD4$^+$ T cell subsets have been associated with autoantibody production in subjects with autoimmune disease (35, 44–48). There may be a spectrum of tolerance controls in healthy individuals, with subjects at the lower end

being more likely to develop autoantibodies, and potentially also to produce bnAbs in the context of HIV-1 infection.

We previously demonstrated that two bnAbs (2F5, 4E10) directed at the HIV-1 Env gp41 neutralizing site near the viral membrane are autoreactive (13, 83), and in bnAb antibody heavy and light chain knock-in mice, both bnAbs were shown to be controlled by multiple immune tolerance mechanisms (21, 22, 24, 84, 85). The observations of bnAb autoreactivity prompted the hypothesis that patients with systemic lupus erythematosus (SLE) will be able to make bnAbs more readily than others during chronic HIV-1 infection (15). We recently described an individual with both HIV-1 infection and SLE who had serum anti-dsDNA and bnAb activity (86), and remarkably, an isolated CD4 binding site bnAb (CH98) from this individual cross-reacted with dsDNA—thus, providing direct evidence that bnAbs and SLE autoantibodies can be derived from similar autoreactive pools of B cells and may be similarly regulated (86). Regardless of the mechanisms involved, the presence of autoantibodies in plasma is an indication of HIV-1-associated breaks in immune tolerance, and our finding of decreased frequency of $CD25^+$ $Foxp3^+$ regulatory $CD4^+$ T cell subsets in HIV-1-infected individuals who make bnAbs, together with increased expression of PD-1 on regulatory $CD4^+$ T cell populations, suggests a mechanism of release of peripheral immune tolerance controls (35).

The studies presented here raise several hypotheses. First, our data suggest that continued immunization of animals and humans with HIV-1 envelopes in the absence of modifying host immune tolerance mechanisms or mimicking other HIV-1-induced immune perturbations will be unlikely to induce mature bnAbs. New vaccination strategies for amplifying antibody responses by limiting immune tolerance controls of antibody responses to bnAb Env epitopes will likely be needed. Such vaccination strategies are already being tested in the setting of cancer vaccines to augment host anti-cancer T cell responses (87). Temporary breaks in tolerance may be mediated by strong adjuvants, since we have shown that peripheral anergy of bnAb-producing B cells can be broken in bnAb V_HDJ_H/V_LJ_L knock-in mice by immunization with an Env subunit with a TLR4 agonist (23). Furthermore, TLR9 agonists can boost Tfh differentiation while blocking Tfr, thereby skewing the Tfr/Tfh ratio in favor of Tfh (88, 89).

Second, based on the continuum of bnAb responses made after HIV-1 infection ((7) and the present study), it is possible that a strategy that succeeds in transiently breaking immune tolerance in the setting of HIV-1 Env immunization may only induce bnAbs in some individuals. Some bnAbs are restricted during early B-cell development at the first tolerance checkpoint in bone marrow due to germline B-cell receptor (BCR) autoreactivity, resulting in fewer bnAb precursors before vaccination (21, 22, 24, 84, 85), while other bnAb germline BCRs are not autoreactive, and autoreactivity is only acquired in the periphery during affinity maturation (90). BnAbs with long third heavy chain complementarity determining regions that do emerge in HIV-1 infection appear to be rare by virtue of tolerance mechanisms that reduce their precursor frequency (12, 26, 91). Recent data have demonstrated that one form of immune tolerance is continued accumulation of somatic mutations in autoantibody (92) and bnAb B cell lineages (23) that can lead to reduction in BCR antibody autoreactivity and, in the case of bnAb development, can reduce bnAb

activity. It is important to note that not all bnAbs that eventually are made in HIV-1-infected individuals are autoreactive (93, 94), and immunization strategies are being developed to select and drive such subdominant B-cell lineages (12, 16, 95).

Finally, low affinity BCR autoreactivity can be a normal component of the human B-cell response (96, 97). Thus, transient manipulation of the germinal center response to augment persistent responses of normal autoreactive pools of B cells without permanently breaking systemic immune tolerance to induce desired bnAb B-cell clonal lineages is plausible.

Supplementary Material

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References

1. Fauci AS, Marston HD. Ending AIDS--is an HIV vaccine necessary? N Engl J Med. 2014; 370:495–498. [PubMed: 24499210]
2. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol Rev. 2013; 254:225–244. [PubMed: 23772623]
3. Burton DR, Mascola JR. Antibody responses to envelope glycoproteins in HIV-1 infection. Nat Immunol. 2015; 16:571–576. [PubMed: 25988889]
4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. N Engl J Med. 2009; 361:2209–2220. [PubMed: 19843557]
5. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, mclinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, Moody MA, Bonsignori M, Ochsenbauer C, Kappes J, Tang H, Greene K, Gao H, Labranche CC, Andrews C, Polonis VR, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Self SG, Berman PW, Francis D, Sinangil F, Lee C, Tartaglia J, Robb ML, Haynes BF, Michael NL, Kim JH. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. Journal of Infectious Diseases. 2012; 206:431–441. [PubMed: 22634875]
6. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, Koblin BA, Buchbinder SP, Keefer MC, Tomaras GD, Frahm N, Hural J, Anude C, Graham BS, Enama ME, Adams E, DeJesus E, Novak RM, Frank I, Bentley C, Ramirez S, Fu R, Koup RA, Mascola JR, Nabel GJ, Montefiori DC, Kublin J, McElrath MJ, Corey L, Gilbert PB. HVTN 505 Study Team. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med. 2013; 369:2083–2092. [PubMed: 24099601]

7. Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS. 2014; 28:163–169. [PubMed: 24361678]

8. Doria-Rose NA, Klein RM, Daniels MG, O'Dell S, Nason M, Lapedes A, Bhattacharya T, Migueles SA, Wyatt RT, Korber BT, Mascola JR, Connors M. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol. 2010; 84:1631–1636. [PubMed: 19923174]

9. Sather DN, Armann J, Ching LK, Mavrantoni A, Sellhorn G, Caldwell Z, Yu X, Wood B, Self S, Kalams S, Stamatatos L. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol. 2009; 83:757–769. [PubMed: 18987148]

10. Walker LM, Simek MD, Priddy F, Gach JS, Wagner D, Zwick MB, Phogat SK, Poignard P, Burton DR. A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. PLoS Pathog. 2010; 6:e1001028. [PubMed: 20700449]

11. Gray ES, Madiga MC, Hermanus T, Moore PL, Wibmer CK, Tumba NL, Werner L, Mlisana K, Sibeko S, Williamson C, Abdool Karim SS, Morris L. the CAPRISA002 Study Team. The Neutralization Breadth of HIV-1 Develops Incrementally over Four Years and Is Associated with CD4+ T Cell Decline and High Viral Load during Acute Infection. J Virol. 2011; 85:4828–4840. [PubMed: 21389135]

12. Haynes BF, Verkoczy L. AIDS/HIV. Host controls of HIV neutralizing antibodies. Science. 2014; 344:588–589. [PubMed: 24812389]

13. Haynes BF. Cardiolipin Polyspecific Autoreactivity in Two Broadly Neutralizing HIV-1 Antibodies. Science. 2005; 308:1906–1908. [PubMed: 15860590]

14. Mouquet H, Scheid JF, Zoller MJ, Krogsgaard M, Ott RG, Shukair S, Artyomov MN, Pietzsch J, Connors M, Pereyra F, Walker BD, Ho DD, Wilson PC, Seaman MS, Eisen HN, Chakraborty AK, Hope TJ, Ravetch JV, Wardemann H, Nussenzweig MC. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. Nature. 2010; 467:591–595. [PubMed: 20882016]

15. Haynes BF, Moody MA, Verkoczy L, Kelsoe G, Alam SM. Antibody polyspecificity and neutralization of HIV-1: a hypothesis. Hum Antibodies. 2005; 14:59–67. [PubMed: 16720975]

16. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nat Biotechnol. 2012; 30:423–433. [PubMed: 22565972]

17. Batista FD, Neuberger MS. Affinity dependence of the B cell response to antigen: a threshold, a ceiling, and the importance of off-rate. Immunity. 1998; 8:751–759. [PubMed: 9655489]

18. Zhang J, Shakhnovich EI, Chakraborty AK. Optimality of mutation and selection in germinal centers. PLoS Comput Biol. 2010; 6:e1000800. [PubMed: 20532164]

19. Kepler TB, Perelson AS. Somatic hypermutation in B cells: an optimal control treatment. J Theor Biol. 1993; 164:37–64. [PubMed: 8264243]

20. Chen C, Nagy Z, Radic MZ, Hardy RR, Huszar D, Camper SA, Weigert M. The site and stage of anti-DNA B-cell deletion. Nature. 1995; 373:252–255. [PubMed: 7816141]

21. Verkoczy L, Diaz M, Holl TM, Ouyang Y-B, Bouton-Verville H, Alam SM, Kelsoe G, Haynes BF. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. Proceedings of the National Academy of Sciences. 2010; 107:181–186.

22. Verkoczy L, Chen Y, Bouton-Verville H, Zhang J, Diaz M, Hutchinson J, Ouyang Y-B, Alam SM, Holl TM, Hwang K-K, Kelsoe G, Haynes BF. Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH × VL knockin mice reveals multiple tolerance controls. 2011; 187:3785–3797.

23. Verkoczy L, Chen Y, Zhang J, Bouton-Verville H, Newman A, Lockwood B, Scearce RM, Montefiori DC, Dennison SM, Xia S-M, Hwang K-K, Alam SM, Haynes BF. Induction of HIV-1 broad neutralizing antibodies in 2F5 knock-in mice: selection against membrane proximal external region-associated autoreactivity limits T-dependent responses. 2013; 191:2538–2550.

24. Chen Y, Zhang J, Hwang K-K, Bouton-Verville H, Xia S-M, Newman A, Ouyang Y-B, Haynes BF, Verkoczy L. Common tolerance mechanisms, but distinct cross-reactivities associated with gp41

and lipids, limit production of HIV-1 broad neutralizing antibodies 2F5 and 4E10. J Immunol. 2013; 191:1260–1275. [PubMed: 23825311]

25. Shiokawa S, Mortari F, Lima JO, Nuñez C, Bertrand FE, Kirkham PM, Zhu S, Dasanayake AP, Schroeder HW. IgM heavy chain complementarity-determining region 3 diversity is constrained by genetic and somatic mechanisms until two months after birth. 1999; 162:6060–6070.
26. Meffre E, Milili M, Blanco-Betancourt C, Antunes H, Nussenzweig MC, Schiff C. Immunoglobulin heavy chain expression shapes the B cell receptor repertoire in human B cell development. J Clin Invest. 2001; 108:879–886. [PubMed: 11560957]
27. Canoso RT, Zon LI, Groopman JE. Anticardiolipin antibodies associated with HTLV-III infection. Br J Haematol. 1987; 65:495–498. [PubMed: 3580308]
28. Kopelman RG, Zolla-Pazner S. Association of human immunodeficiency virus infection and autoimmune phenomena. Am J Med. 1988; 84:82–88. [PubMed: 3257353]
29. Morrow WJ, Isenberg DA, Sobol RE, Stricker RB, Kieber-Emmons T. AIDS virus infection and autoimmunity: a perspective of the clinical, immunological, and molecular origins of the autoallergic pathologies associated with HIV disease. Clin Immunol Immunopathol. 1991; 58:163–180. [PubMed: 1985795]
30. Zandman-Goddard G, Shoenfeld Y. HIV and autoimmunity. Autoimmun Rev. 2002; 1:329–337. [PubMed: 12848988]
31. Reveille JD. The changing spectrum of rheumatic disease in human immunodeficiency virus infection. Semin Arthritis Rheum. 2000; 30:147–166. [PubMed: 11124280]
32. Sène D, Piette J-C, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and infections. Autoimmun Rev. 2008; 7:272–277. [PubMed: 18295729]
33. Iordache L, Launay O, Bouchaud O, Jeantils V, Goujard C, Boue F, Cacoub P, Hanslik T, Mahr A, Lambotte O, Fain O. Associated authors. Autoimmune diseases in HIV-infected patients: 52 cases and literature review. Autoimmun Rev. 2014; 13:850–857. [PubMed: 24747058]
34. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, Su LF, Cubas R, Davis MM, Sette A, Haddad EK, Poignard P, Crotty S. A. V. I. P. C. P. I. International. Human circulating PD-1+CXCR3−CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity. 2013; 39:758–769. [PubMed: 24035365]
35. Wing JB, Sakaguchi S. Foxp3^{+} T(reg) cells in humoral immunity. Int Immunol. 2014; 26:61–69. [PubMed: 24324208]
36. Lim HW, Hillsamer P, Kim CH. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. J Clin Invest. 2004; 114:1640–1649. [PubMed: 15578096]
37. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, Wang Y-H, Lim H, Reynolds JM, Zhou X-H, Fan H-M, Liu Z-M, Neelapu SS, Dong C. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nat Med. 2011; 17:983–988. [PubMed: 21785430]
38. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, Srivastava M, Divekar DP, Beaton L, Hogan JJ, Fagarasan S, Liston A, Smith KGC, Vinuesa CG. Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med. 2011; 17:975–982. [PubMed: 21785433]
39. Wollenberg I, Agua-Doce A, Hernández A, Almeida C, Oliveira VG, Faro J, Graca L. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. J Immunol. 2011; 187:4553–4560. [PubMed: 21984700]
40. Sage PT, Paterson AM, Lovitch SB, Sharpe AH. The coinhibitory receptor CTLA-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells. Immunity. 2014; 41:1026–1039. [PubMed: 25526313]
41. Sage PT, Francisco LM, Carman CV, Sharpe AH. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. Nat Immunol. 2013; 14:152–161. [PubMed: 23242415]
42. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. Immunity. 2014; 41:1013–1025. [PubMed: 25526312]

43. Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med. 1983; 309:453–458. [PubMed: 6224088]
44. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev. 2006; 212:8–27. [PubMed: 16903903]
45. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. Immunity. 2013; 38:414–423. [PubMed: 23521883]
46. Morita R, Schmitt N, Bentebibel S-E, Ranganathan R, Bourdery L, Zurawski G, Foucat E, Dullaers M, Oh S, Sabzghabaei N, Lavecchio EM, Punaro M, Pascual V, Banchereau J, Ueno H. Human Blood CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion. Immunity. 2011; 34:108–121. [PubMed: 21215658]
47. Ma CS, Deenick EK, Batten M, Tangye SG. The origins, function, and regulation of T follicular helper cells. Journal of Experimental Medicine. 2012; 209:1241–1253. [PubMed: 22753927]
48. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, Manku H, Vyse TJ, Roncador G, Huttley GA, Goodnow CC, Vinuesa CG, Cook MC. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. 2010; 62:234–244.
49. Velu V, Shetty RD, Larsson M, Shankar EM. Role of PD-1 co-inhibitory pathway in HIV infection and potential therapeutic options. Retrovirology. 2015; 12:14. [PubMed: 25756928]
50. Gianchecchi E, Delfino DV, Fierabracci A. Recent insights into the role of the PD-1/PD-L1 pathway in immunological tolerance and autoimmunity. Autoimmun Rev. 2013; 12:1091–1100. [PubMed: 23792703]
51. Chang J-H, Chung Y. Regulatory T cells in B cell follicles. Immune Netw. 2014; 14:227–236. [PubMed: 25360073]
52. Sage PT, Sharpe AH. T follicular regulatory cells in the regulation of B cell responses. Trends in Immunology. 2015; 36:410–418. [PubMed: 26091728]
53. Sage PT, Alvarez D, Godec J, von Andrian UH, Sharpe AH. Circulating T follicular regulatory and helper cells have memory-like properties. J Clin Invest. 2014; 124:5191–5204. [PubMed: 25347469]
54. Wei, Y., Feng, J., Hou, Z., Wang, XM., Yu, D. Methods in Molecular Biology. Vol. 1291. Springer New York; New York, NY: 2015. T follicular Helper Cells; p. 199-207.
55. Wang L, Qiu J, Yu L, Hu X, Zhao P, Jiang Y. Increased numbers of CD5+CD19+CD1dhighIL-10+ Bregs, CD4+Foxp3+ Tregs, CD4+CXCR5+Foxp3+ follicular regulatory T (TFR) cells in CHB or CHC patients. J Transl Med. 2014; 12:4178.
56. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, Wherry EJ. Molecular and transcriptional basis of $CD4^+$ T cell dysfunction during chronic infection. Immunity. 2014; 40:289–302. [PubMed: 24530057]
57. Thornton AM, Piccirillo CA, Shevach EM. Activation requirements for the induction of CD4+CD25+ T cell suppressor function. Eur J Immunol. 2004; 34:366–376. [PubMed: 14768041]
58. Huang C-T, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, Powell JD, Pardoll DM, Drake CG, Vignali DAA. Role of LAG-3 in regulatory T cells. Immunity. 2004; 21:503–513. [PubMed: 15485628]
59. Rioux JD, Goyette P, Vyse TJ, Hammarström L, Fernando MMA, Green T, De Jager PL, Foisy S, Wang J, de Bakker PIW, Leslie S, McVean G, Padyukov L, Alfredsson L, Annese V, Hafler DA, Pan-Hammarström Q, Matell R, Sawcer SJ, Compston AD, Cree BAC, Mirel DB, Daly MJ, Behrens TW, Klareskog L, Gregersen PK, Oksenberg JR, Hauser SL. International MHC, Autoimmunity Genetics Network. Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. Proceedings of the National Academy of Sciences. 2009; 106:18680–18685.
60. Lifson JD, Piatak MJ, Rossio JL, Bess JJ, Chertova E, Schneider D, Kiser R, Coalter V, Poore B, Imming R, Desrosiers RC, Henderson LE, Arthur LO. Whole inactivated SIV virion vaccines with

functional envelope glycoproteins: safety, immunogenicity, and activity against intrarectal challenge. J Med Primatol. 2002; 31:205–216. [PubMed: 12390543]

61. Lifson JD, Rossio JL, Piatak M, Bess J, Chertova E, Schneider DK, Coalter VJ, Poore B, Kiser RF, Imming RJ, Scarzello AJ, Henderson LE, Alvord WG, Hirsch VM, Benveniste RE, Arthur LO. Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccines with conformationally and functionally intact envelope glycoproteins. AIDS Res Hum Retroviruses. 2004; 20:772–787. [PubMed: 15307924]
62. Tong T, Crooks ET, Osawa K, Robinson JE, Barnes M, Apetrei C, Binley JM. Multi-parameter exploration of HIV-1 virus-like particles as neutralizing antibody immunogens in guinea pigs, rabbits and macaques. Virology. 2014; 456–457:55–69.
63. Willey RL, Byrum R, Piatak M, Kim YB, Cho MW, Rossio JL, Bess J, Igarashi T, Endo Y, Arthur LO, Lifson JD, Martin MA. Control of viremia and prevention of simian-human immunodeficiency virus-induced disease in rhesus macaques immunized with recombinant vaccinia viruses plus inactivated simian immunodeficiency virus and human immunodeficiency virus type 1 particles. J Virol. 2003; 77:1163–1174. [PubMed: 12502833]
64. Kovacs JM, Nkolola JP, Peng H, Cheung A, Perry J, Miller CA, Seaman MS, Barouch DH, Chen B. HIV-1 envelope trimer elicits more potent neutralizing antibody responses than monomeric gp120. Proceedings of the National Academy of Sciences. 2012; 109:12111–12116.
65. Bricault CA, Kovacs JM, Nkolola JP, Yusim K, Giorgi EE, Shields JL, Perry J, Lavine CL, Cheung A, Ellingson-Strouss K, Rademeyer C, Gray GE, Williamson C, Stamatatos L, Seaman MS, Korber BT, Chen B, Barouch DH, Silvestri G. Ed. A multivalent clade C HIV-1 Env trimer cocktail elicits a higher magnitude of neutralizing antibodies than any individual component. J Virol. 2015; 89:2507–2519. [PubMed: 25540368]
66. Sanders RW, van Gils MJ, Derking R, Sok D, Ketas TJ, Burger JA, Ozorowski G, Cupo A, Simonich C, Goo L, Arendt H, Kim HJ, Lee J-H, Pugach P, Williams M, Debnath G, Moldt B, van Breemen MJ, Isik G, Medina-Ramírez M, Back JW, Koff WC, Julien J-P, Rakasz EG, Seaman MS, Guttman M, Lee KK, Klasse PJ, Labranche C, Schief WR, Wilson IA, Overbaugh J, Burton DR, Ward AB, Montefiori DC, Dean H, Moore JP. HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science. 2015; 349:aac4223–aac4223. [PubMed: 26089353]
67. Gray ES, Taylor N, Wycuff D, Moore PL, Tomaras GD, Wibmer CK, Puren A, DeCamp A, Gilbert PB, Wood B, Montefiori DC, Binley JM, Shaw GM, Haynes BF, Mascola JR, Morris L. Antibody specificities associated with neutralization breadth in plasma from human immunodeficiency virus type 1 subtype C-infected blood donors. J Virol. 2009; 83:8925–8937. [PubMed: 19553335]
68. The INSIGHT START Study Group. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med. 2015; 373:795–807. [PubMed: 26192873]
69. Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, Baba TW, Ruprecht RM, Kupfer A. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med. 1995; 1:129–134. [PubMed: 7585008]
70. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. Blood. 2013; 121:29–37. [PubMed: 23043072]
71. Moore PL, Williamson C, Morris L. Virological features associated with the development of broadly neutralizing antibodies to HIV-1. Trends Microbiol. 2015; 23:204–211. [PubMed: 25572881]
72. Crotty S. T follicular helper cell differentiation, function, and roles in disease. Immunity. 2014; 41:529–542. [PubMed: 25367570]
73. Harker JA, Lewis GM, Mack L, Zuniga EI. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. Science. 2011; 334:825–829. [PubMed: 21960530]
74. Fahey LM, Wilson EB, Elsaesser H, Fistonich CD, McGavern DB, Brooks DG. Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. J Exp Med. 2011; 208:987–999. [PubMed: 21536743]
75. Lindqvist M, van Lunzen J, Soghoian DZ, Kuhl BD, Ranasinghe S, Kranias G, Flanders MD, Cutler S, Yudanin N, Muller MI, Davis I, Farber D, Hartjen P, Haag F, Alter G, Schulze zur

Wiesch J, Streeck H. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. J Clin Invest. 2012; 122:3271–3280. [PubMed: 22922259]

76. Perreau M, Savoye A-L, De Crignis E, Corpataux J-M, Cubas R, Haddad EK, De Leval L, Graziosi C, Pantaleo G. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. Journal of Experimental Medicine. 2013; 210:143–156. [PubMed: 23254284]
77. Cubas RA, Mudd JC, Savoye A-L, Perreau M, van Grevenynghe J, Metcalf T, Connick E, Meditz A, Freeman GJ, Abesada-Terk G, Jacobson JM, Brooks AD, Crotty S, Estes JD, Pantaleo G, Lederman MM, Haddad EK. Inadequate T follicular cell help impairs B cell immunity during HIV infection. Nat Med. 2013; 19:494–499. [PubMed: 23475201]
78. Cohen K, Altfeld M, Alter G, Stamatatos L. Early preservation of CXCR5+ PD-1+ helper T cells and B cell activation predict the breadth of neutralizing antibody responses in chronic HIV-1 infection. J Virol. 2014; 88:13310–13321. [PubMed: 25210168]
79. Chowdhury A, Del Rio Estrada PM, Del Rio PME, Tharp GK, Trible RP, Amara RR, Chahroudi A, Reyes-Teran G, Bosinger SE, Silvestri G. Decreased T Follicular Regulatory Cell/T Follicular Helper Cell (TFH) in Simian Immunodeficiency Virus-Infected Rhesus Macaques May Contribute to Accumulation of TFH in Chronic Infection. J Immunol. 2015; 195:3237–3247. [PubMed: 26297764]
80. Miles B, Miller SM, Folkvord JM, Kimball A, Chamanian M, Meditz AL, Arends T, McCarter MD, Levy DN, Rakasz EG, Skinner PJ, Connick E. Follicular regulatory T cells impair follicular T helper cells in HIV and SIV infection. Nat Commun. 2015; 6:8608. [PubMed: 26482032]
81. Colineau L, Rouers A, Yamamoto T, Xu Y, Urrutia A, Pham H-P, Cardinaud S, Samri A, Dorgham K, Coulon P-G, Cheynier R, Hosmalin A, Oksenhendler E, Six A, Kelleher AD, Zaunders J, Koup RA, Autran B, Moris A, Graff-Dubois S, Unutmaz D. Ed. HIV-Infected Spleens Present Altered Follicular Helper T Cell (Tfh) Subsets and Skewed B Cell Maturation. PLoS ONE. 2015; 10:e0140978. [PubMed: 26501424]
82. Aloulou M, Carr EJ, Gador M, Bignon A, Liblau RS, Fazilleau N, Linterman MA. Follicular regulatory T cells can be specific for the immunizing antigen and derive from naive T cells. Nat Commun. 2016; 7:10579. [PubMed: 26818004]
83. Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, Kepler TB, Alam SM, Cain DW, Spicer L, VandeBerg JL, Haynes BF, Kelsoe G. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. Journal of Experimental Medicine. 2013; 210:241–256. [PubMed: 23359068]
84. Verkoczy L, Moody MA, Holl TM, Bouton-Verville H, Scearce RM, Hutchinson J, Alam SM, Kelsoe G, Haynes BF, Unutmaz D. Functional, non-clonal IgMa-restricted B cell receptor interactions with the HIV-1 envelope gp41 membrane proximal external region. PLoS ONE. 2009; 4:e7215. [PubMed: 19806186]
85. Doyle-Cooper C, Hudson KE, Cooper AB, Ota T, Skog P, Dawson PE, Zwick MB, Schief WR, Burton DR, Nemazee D. Immune tolerance negatively regulates B cells in knock-in mice expressing broadly neutralizing HIV antibody 4E10. J Immunol. 2013; 191:3186–3191. [PubMed: 23940276]
86. Bonsignori M, Wiehe K, Grimm SK, Lynch R, Yang G, Kozink DM, Perrin F, Cooper AJ, Hwang K-K, Chen X, Liu M, McKee K, Parks RJ, Eudailey J, Wang M, Clowse M, Criscione-Schreiber LG, Moody MA, Ackerman ME, Boyd SD, Gao F, Kelsoe G, Verkoczy L, Tomaras GD, Kepler TB, Montefiori DC, Mascola JR, Haynes BF. An autoreactive antibody from an SLE/HIV-1 individual broadly neutralizes HIV-1. J Clin Invest. 2014; 124:1835–1843. [PubMed: 24614107]
87. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015; 348:56–61. [PubMed: 25838373]
88. Chakarov S, Fazilleau N. Monocyte-derived dendritic cells promote T follicular helper cell differentiation. EMBO Mol Med. 2014; 6:590–603. [PubMed: 24737871]
89. Rookhuizen DC, DeFranco AL. Toll-like receptor 9 signaling acts on multiple elements of the germinal center to enhance antibody responses. Proceedings of the National Academy of Sciences. 2014; 111:E3224–33.
90. Liao H-X, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, Fire AZ, Roskin KM, Schramm CA, Zhang Z, Zhu J, Shapiro L, Mullikin JC, Gnanakaran S, Hraber P, Wiehe K, Kelsoe G, Yang G,

Xia S-M, Montefiori DC, Parks R, Lloyd KE, Scearce RM, Soderberg KA, Cohen M, Kamanga G, Louder MK, Tran LM, Chen Y, Cai F, Chen S, Moquin S, Du X, Joyce MG, Srivatsan S, Zhang B, Zheng A, Shaw GM, Hahn BH, Kepler TB, Korber BTM, Kwong PD, Mascola JR, Haynes BF. NISC Comparative Sequencing Program. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013; 496:469–476. [PubMed: 23552890]

91. Briney BS, Willis JR, Crowe JE, Hoshino Y. Human peripheral blood antibodies with long HCDR3s are established primarily at original recombination using a limited subset of germline genes. PLoS ONE. 2012; 7:e36750. [PubMed: 22590602]
92. Sabouri Z, Schofield P, Horikawa K, Spierings E, Kipling D, Randall KL, Langley D, Roome B, Vazquez-Lombardi R, Rouet R, Hermes J, Chan TD, Brink R, Dunn-Walters DK, Christ D, Goodnow CC. Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity. Proceedings of the National Academy of Sciences. 2014; 111:E2567–75.
93. Walker LM, Phogat SK, Chan-Hui P-Y, Wagner D, Phung P, Goss JL, Wrin T, Simek MD, Fling S, Mitcham JL, Lehrman JK, Priddy FH, Olsen OA, Frey SM, Hammond PW, Kaminsky S, Zamb T, Moyle M, Koff WC, Poignard P, Burton DR. Protocol G Principal Investigators. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009; 326:285–289. [PubMed: 19729618]
94. Bonsignori M, Hwang KK, Chen X, Tsao CY, Morris L, Gray E, Marshall DJ, Crump JA, Kapiga SH, Sam NE, Sinangil F, Pancera M, Yongping Y, Zhang B, Zhu J, Kwong PD, O'Dell S, Mascola JR, Wu L, Nabel GJ, Phogat S, Seaman MS, Whitesides JF, Moody MA, Kelsoe G, Yang X, Sodroski J, Shaw GM, Montefiori DC, Kepler TB, Tomaras GD, Alam SM, Haynes BF. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J Virol. 2011; 85:9998–10009. [PubMed: 21795340]
95. McGuire AT, Dreyer AM, Carbonetti S, Lippy A, Glenn J, Scheid JF, Mouquet H, Stamatatos L. HIV antibodies. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. Science. 2014; 346:1380–1383. [PubMed: 25504724]
96. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003; 301:1374–1377. [PubMed: 12920303]
97. Tiller T, Tsuiji M, Yurasov S, Velinzon K, Nussenzweig MC, Wardemann H. Autoreactivity in human IgG+ memory B cells. Immunity. 2007; 26:205–213. [PubMed: 17306569]

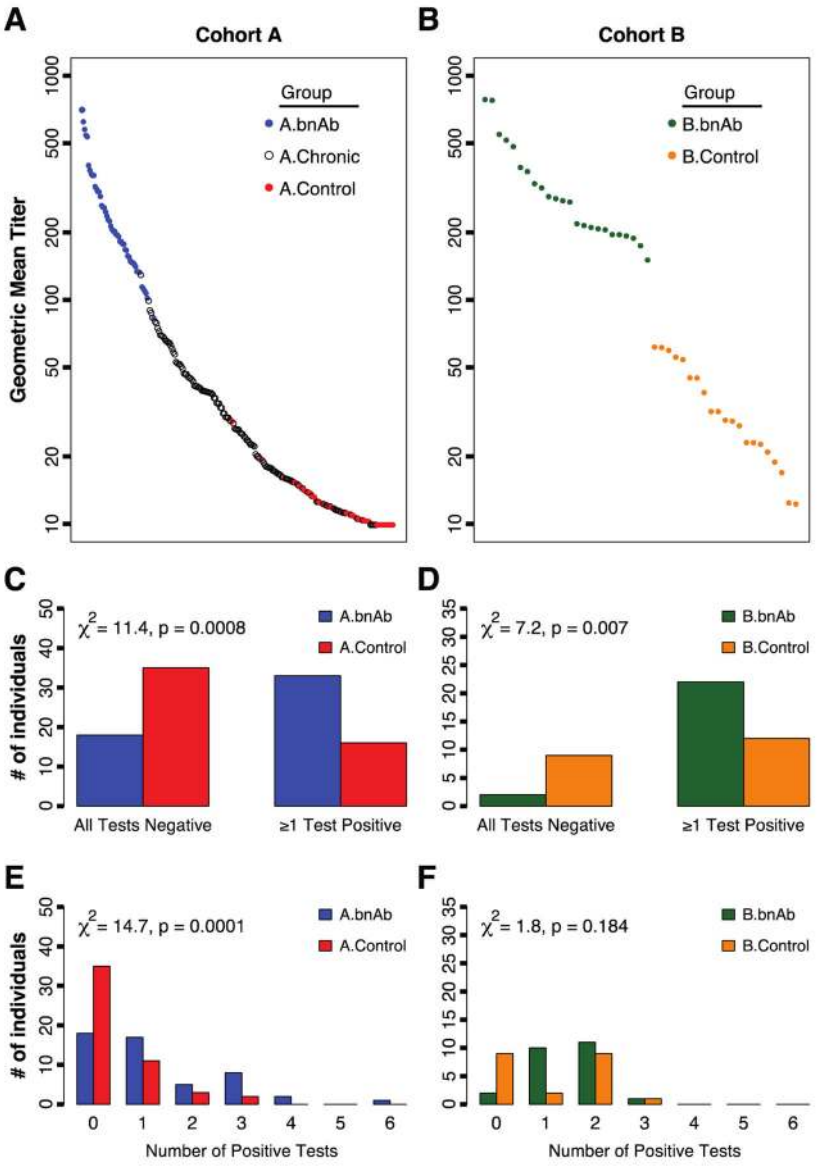

Figure 1.

Neutralization and autoantibody testing of HIV-1-infected individuals. HIV-1 neutralization data are shown as geometric mean titer for a panel of isolates for Cohort A (**A**) and B (**B**). Individuals with the highest (bnAb) and lowest (Control) HIV-1 neutralization for each cohort were tested in autoantibody assays. **Panels C and D** compare the frequency of individuals in the bnAb and control groups with any positive result for Cohorts A and B, respectively. The number of positive autoantibody results is shown for Cohort A (**panel E**) and B (**panel F**).

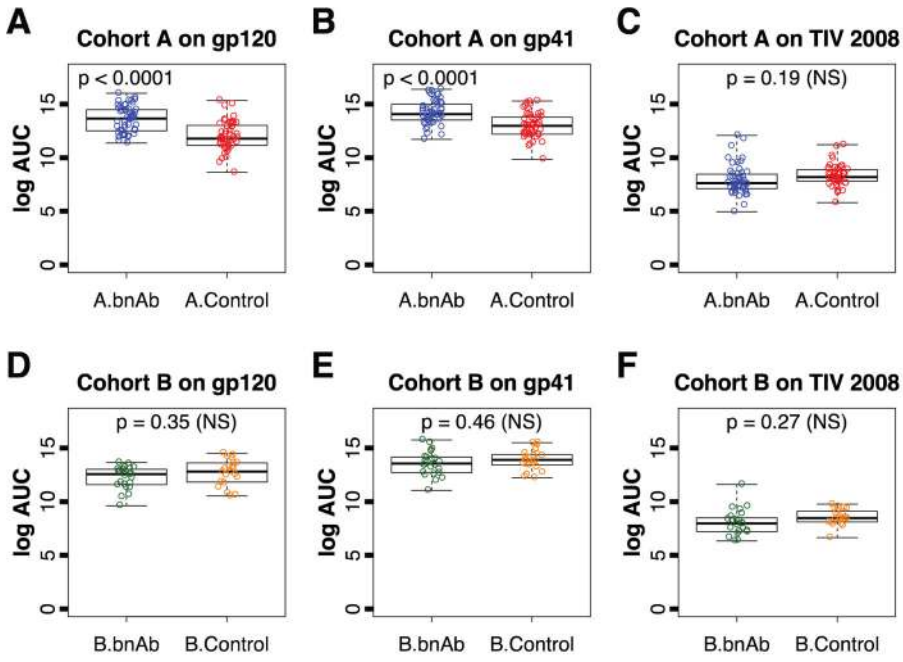

Figure 2.

Comparison of plasma antibody responses. When compared with the A.Control group, the A.bnAb group had higher binding to HIV-1 Env gp120 (**A**) and gp41 (**B**) but similar binding (**C**) to trivalent inactivated influenza vaccine for 2008 (TIV 2008). Binding was similar between the B.bnAb and B.Control groups for the same three antigens (**D – F**). Each symbol represents data from an individual subject; group medians, range, and quartiles are shown.

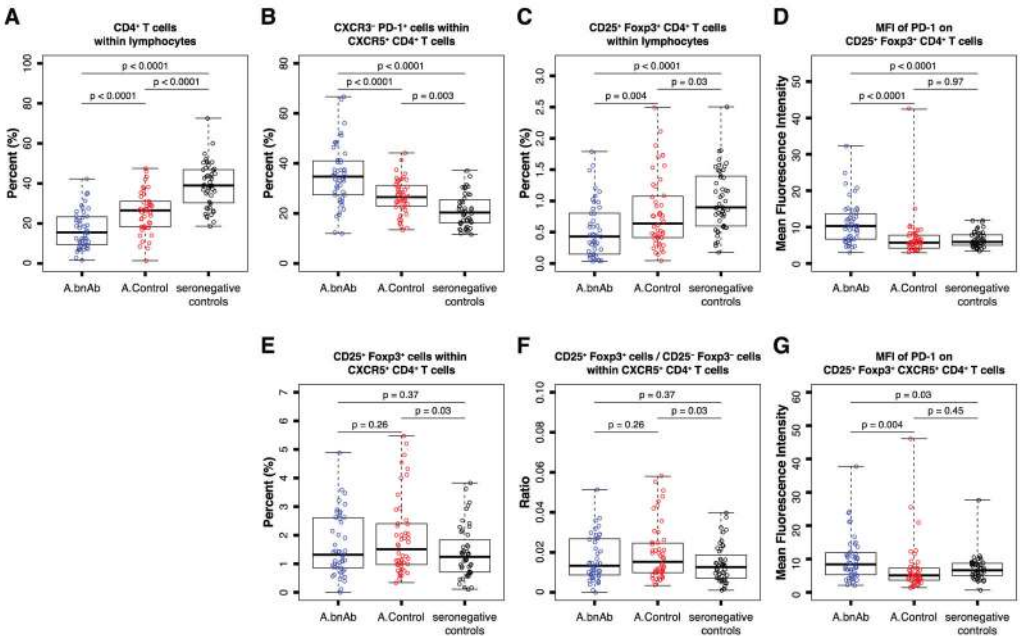

Figure 3.

T cell subsets in Cohort A subjects with and without bnAbs and matched HIV-1-seronegative controls. Total CD4$^+$ T cell frequency within lymphocytes was lower in Cohort A HIV-1-infected individuals vs. HIV-1-seronegative controls and was lowest in the A.bnAb group (**A**). Resting memory T follicular helper (mTfh) cells were elevated in Cohort A HIV-1-infected individuals vs. HIV-seronegative controls, and were highest in the A.bnAb group (**B**). CD4$^+$ Treg cell frequency was lowest in the A.bnAb group (**C**). PD-1 MFI on CD4$^+$ Treg cells was highest in the A.bnAb group (**D**). The proportion of Tfr cells within circulating CD4$^+$ follicular-phenotype T cells in the A.bnAb group did not differ significantly from that in the A.Control or seronegative groups (**E**). The Tfr/Tfh ratio, defined as

$$\mathrm{Tfr/Tfh} = \frac{\%\ \mathrm{CD25^+\ Foxp3^+\ cells\ within\ CXCR5^+\ CD4^+\ T\ cells}}{\%\ \mathrm{non\text{-}\ CD25^+\ Foxp3^+\ cells\ within\ CXCR5^+\ CD4^+\ T\ cells}}$$

in the A.bnAb group did not differ significantly from that in the A.Control or seronegative groups (**F**). The MFI of PD-1 staining on CD4$^+$ Tfr cells was highest in the A.bnAb group (**G**). Each symbol represents data from an individual subject; group medians, range, and quartiles are shown.

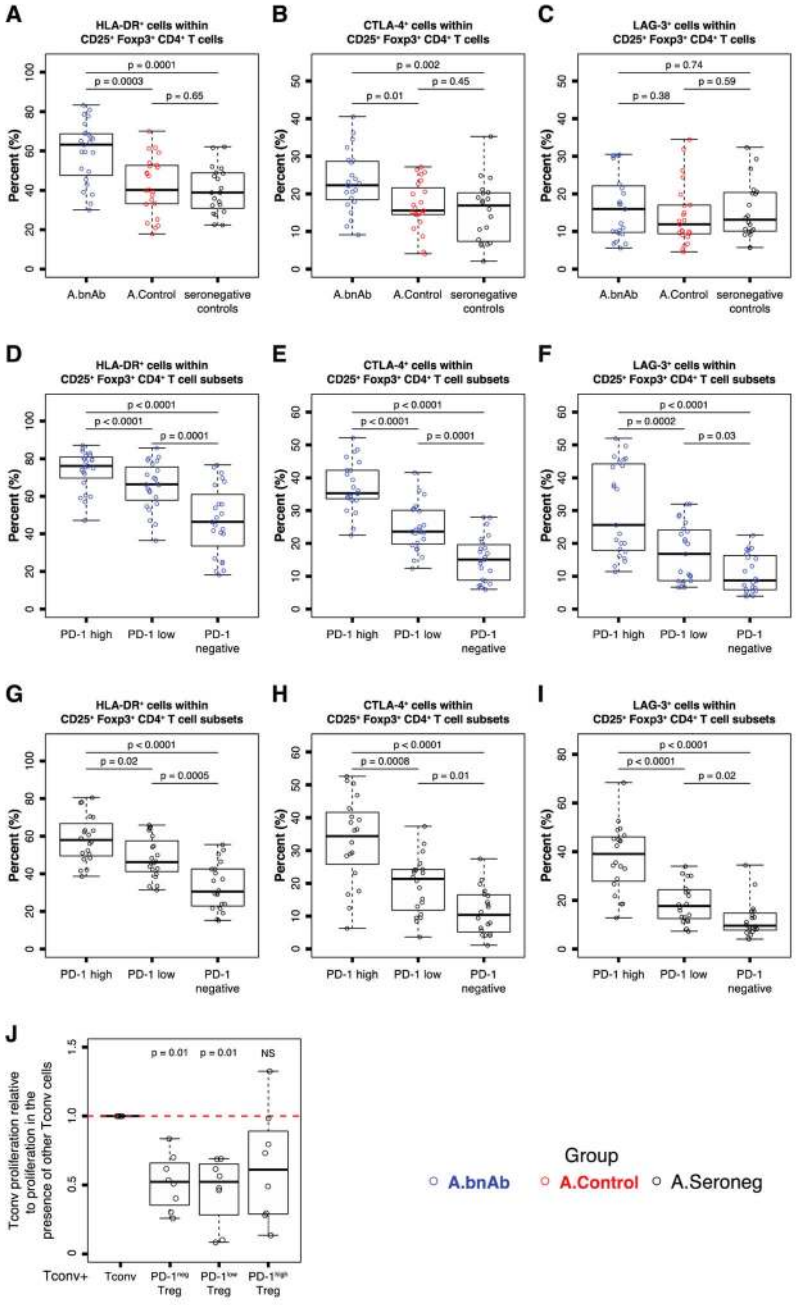

Figure 4.

Phenotypic and functional analysis of CD4$^+$ Treg cells. CD4$^+$ Treg cells expressing HLA-DR were higher in the A.bnAb group vs. the A.Control or seronegative groups (**A**). Total CD4$^+$ Treg cells expressing CTLA-4 were also higher in the A.bnAb group vs. the A.Control or seronegative groups (**B**); no difference between groups was found for CD4$^+$ Treg cells expressing LAG-3 (**C**). In the A.bnAb group, the PD-1^{high} subset of CD4$^+$ Treg cells expressed higher levels of HLA-DR (**D**), CTLA-4 (**E**) and LAG-3 (**F**) than the PD-1^{low} or PD-1 negative subsets of CD4$^+$ Treg cells. The results were similar for the HIV-1 seronegative control group (**G, H** and **I**). In panels **A–I**, symbols represent individual

subjects; group medians, range, and quartiles are shown. In experiments performed with samples from healthy HIV-1-seronegative UK donors, PD-1 negative and PD-1^{low} $CD4^+$ Treg cells suppressed the proliferation of conventional $CD4^+$ T cells (Tconv) compared to that observed in the presence of other conventional $CD4^+$ T cells, whereas PD-1^{high} $CD4^+$ Treg cells did not do so (**J**). In panel **J**, the symbols represent individual subjects; group medians, range and quartiles are shown; and the horizontal dashed red line indicates the level of proliferation of conventional $CD4^+$ T cells in the presence of other conventional $CD4^+$ T cells to which other values were normalized.

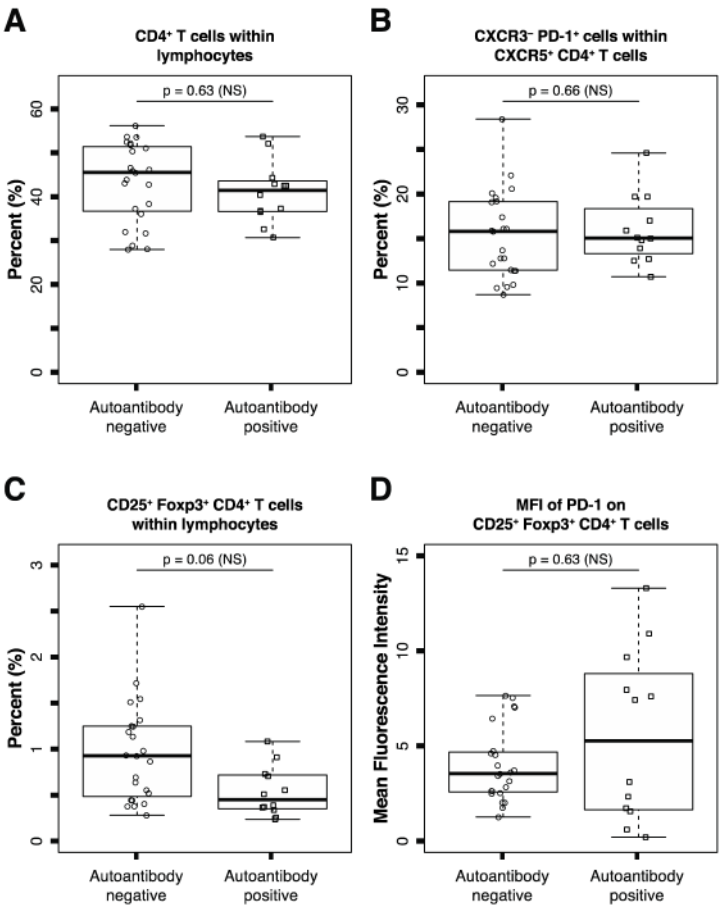

Figure 5.

T cell subsets in healthy, HIV-1-seronegative African individuals with and without autoantibodies. HIV-1-seronegative subjects with and without autoantibodies had similar frequencies of total $CD4^+$ T cells within lymphocytes (**A**). The circulating frequency of resting memory T follicular helper (mTfh) cells was similar in HIV-1-seronegative individuals with and without autoantibodies (**B**). $CD4^+$ Treg cell frequency within lymphocytes in HIV-1-seronegative individuals with autoantibodies did not differ significantly from that in those without autoantibodies (**C**). PD-1 MFI on $CD4^+$ Treg cells in HIV-1-seronegative individuals with autoantibodies did not differ significantly from that in those without autoantibodies (**D**). In all panels, each symbol represents data from an individual subject; group medians, range, and quartiles are shown.