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Limited Humoral and Specific T-Cell Responses After SARS-CoV-2 Vaccination in PWH With Poor Immune Reconstitution

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Background. We analyzed humoral and cellular immune responses induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) messenger RNA (mRNA) vaccines in people with human immunodeficiency virus (HIV; PWH) who had $CD4^+$ T-cell counts <200/μL (HIV<200 group).

Methods. This prospective cohort study included 58 PWH in the HIV<200 group, 36 with $CD4^+$ T-cell counts >500/μL (HIV>500 group), and 33 HIV-1–negative controls (control group). Antibodies against the SARS-CoV-2 spike protein (anti-S immunoglobulin [Ig] G) and the receptor-binding domain (anti-RBD IgG) were quantified before and 4 weeks after the first and the second doses of BNT162b2 or mRNA-1273 (at week 8). Viral neutralization activity and T-cell responses were also determined.

Results. At week 8, anti-S/anti-RBD IgG responses increased in all groups ($P < .001$). Median (interquartile range) anti-S and anti-RBD IgG levels at week 8 were 153.6 (26.4–654.9) and 171.9 (61.8–425.8) binding antibody units (BAU)/mL, respectively, in the HIV<200 group, compared with 245.6 (145–824) and 555.8 (166.4–1751) BAU/mL in the HIV>500 group and 274.7 (193.7–680.4) and 281.6 (181–831.8) BAU/mL in controls ($P < .05$). Neutralizing capacity and specific T-cell immune responses were absent or reduced in 33% of those in the HIV<200 group, compared with 3.7% in the HIV>500 group ($P < .01$).

Conclusions. One-third of PWH with $CD4^+$ T-cell counts <200/μL show low anti-S/anti-RBD IgG levels, reduced in vitro neutralization activity against SARS-CoV-2, and no vaccine-induced T cells after receiving coronavirus disease 2019 mRNA vaccines.

Keywords. anti-RBD IgG; anti-S IgG; neutralizing antibodies; people with HIV; SARS-CoV-2 vaccine.

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), constitutes an unprecedented threat to the global healthcare system. Since the first case reported in Wuhan, China, close to 611 million cases have been documented worldwide, causing >6 million deaths as of September 2022 (https://COVID19.who.int/).

Successful development of safe and effective vaccines has drastically reduced morbidity and mortality rates associated with COVID-19 [1,2]. Both messenger RNA (mRNA) and adenovirus-vectored vaccines show high effectiveness in preventing COVID-19 illness, reducing severe disease and death [1,2]. Levels of binding and neutralizing antibodies directed against the SARS-CoV-2 spike protein (S) and the receptor-binding domain (RBD) are correlated with vaccine efficacy [3–5].

People with human immunodeficiency virus (HIV) (PWH) are at higher risk of developing severe COVID-19 disease [6,7], especially those aged ≥40 years, with $CD4^+$ T-cell counts <200/μL, and/or with ≥3 comorbid conditions [8]. Consequently, PWH with low $CD4^+$ T-cell counts have received priority access to SARS-CoV-2 vaccines in many vaccine rollout programs. However, this population was either excluded or underrepresented in pivotal phase 2/3 vaccine trials as only allowed inclusion of PWH with high $CD4^+$ T-cell counts

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on suppressive antiretroviral therapy (ART) [1]. While PLWH with well-controlled infection showed similar humoral responses compared with the general population [9–12], data on vaccine-induced responses from PWH at higher risk of severe COVID-19 disease are still scarce. This information is essential for the clinical management of this group. Our objective was to characterize humoral and cellular immune responses after SARS-CoV-2 vaccination in PWH with low $CD4^+$ T-cell counts and/or with suboptimal viral suppression.

METHODS

Study Design

A prospective observational single-center cohort study was performed to investigate vaccine-specific immune responses after 2 doses of mRNA-based SARS-CoV-2 vaccination in PWH with distinct levels of immune status, compared with aged-matched HIV-negative controls. The primary end point was the assessment of antibody levels against S and RBD (anti-S and anti-RBD immunoglobulin [Ig] G) after primary (2-dose) vaccination. Antibody levels were measured in all participants at baseline, 21 or 28 days after the first vaccination, and 28 days after the second vaccination (named week 4 and week 8 for consistency). Secondary end points included determination of avidity, IgM/IgA measurements, neutralization activity and vaccine-induced cellular immune responses, which were assessed at baseline and at week 8 in a subgroup of individuals based on representability and sample availability.

All participants included in the study received 2 doses of mRNA SARS-CoV-2 vaccines (21 days apart for BNT162b2 and 28 days apart for mRNA-1273), following the National Vaccination Plan for primary vaccinations. Peripheral blood mononuclear cells and plasma samples were collected at baseline and at weeks 4 and 8. Demographic and clinical data were collected from electronic medical records and during clinical visits.

The study was approved by the institutional ethical review board at Hospital Universitari Germans Trias I Pujol (HUGTiP) in Badalona, Spain (no. PI-21-108). All participants gave written informed consent. The biological biosafety committee approved SARS-CoV-2 experiments at the biosafety level 3 laboratory of the Center for Bioimaging and Comparative Medicine (no. CSB-20-015-M3).

Study Population

In April 2021, once PWH with $CD4^+$ T-cell counts <200/μL were prioritized for vaccination, PWH aged >18 years with records of $CD4^+$ T-cell counts <200/μL within the last year (hereafter, HIV<200 group) under regular care at HUGTiP were contacted. Exclusion criteria included previous SARS-CoV-2 vaccination and known history of SARS-CoV-2 infection (Figure 1, flow diagram). PLWH with $CD4^+$ T-cell counts >500/μL (HIV>500 group) and HIV-1–negative controls, mostly represented by healthcare workers, were included sequentially through opportunistic vaccination at HUGTiP and were matched by age with those in the HIV<200 group.

Enzyme-Linked Immunosorbent Assay

We measured anti-S IgG and anti-RBD IgG antibody levels at enrollment and at week 4 and week 8 in 127 participants who completed follow-up, except for 1 individual in the HIV<200 group, whose results were not valid, and anti-S IgG avidity and anti-S IgM and IgA antibodies in 52 randomly selected participants (26 in the HIV<200, 13 in the HIV>500, and 13 in the control group). Plates were coated overnight at 4°C with the HIS.H8 antibody (Thermo Fisher Scientific) at 2 μg/mL, blocked with phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA; Miltenyi Biotech) for 2 hours at room temperature (RT), washed, and incubated with S, RBD, or nucleocapsid protein (all from Sino Biological) at 1 μg/mL or PBS/1% BSA to estimate sample background. A positive plasma sample was used as the standard, and a pool of 10 SARS-CoV-2 negative plasma samples served as negative control.

Samples were diluted according to their IgG titers (between 1:200 to 1:15,000) and incubated for 1 hour (at RT). Secondary antibodies—horseradish peroxidase–conjugated goat anti-human IgG, goat anti-human IgM, and goat anti-human IgA (all from Jackson ImmunoResearch)—were incubated for 30 minutes. O-Phenylenediamine dihydrochloride (Sigma Aldrich) was added, and the enzymatic reaction was stopped with 2 mol/L sulfuric acid (Sigma Aldrich). Signal was evaluated as the optical density at 492 nm with noise correction at 620 nm. The specific signal for each antigen was determined after subtracting background obtained from antigen-free wells. Results are expressed as arbitrary units (AU) per milliliter, according to the standard [13].

Anti-S IgG avidity was evaluated by enzyme-linked immunosorbent assay. Plates were coated with S (Sino Biological) at 1 μg/mL and were blocked with PBS/1% BSA for 2 hours at RT. Samples were diluted at 0.5 AU/mL and evaluated in quadruplicate for 1 hour at RT. After washing, 2 mol/L guanidine-hydrochloride or PBS were added and incubated for 15 minutes at RT. Bound antibodies were detected using horseradish peroxidase–conjugated goat anti-human IgG (Jackson ImmunoResearch), and plates were revealed using O-phenylenediamine dihydrochloride (Sigma Aldrich). The enzymatic reaction was stopped with 2 mol/L sulfuric acid (Sigma Aldrich), and the signal was evaluated as optical density at 492 nm with noise correction at 620 nm. Anti-S IgG avidity was calculated as the ratio between the mean signal obtained with and without guanidine treatment.

Viral Isolation and Titration

SARS-CoV-2 was isolated from a nasopharyngeal swab sample collected in March 2020 in Spain and propagated for 2 passages

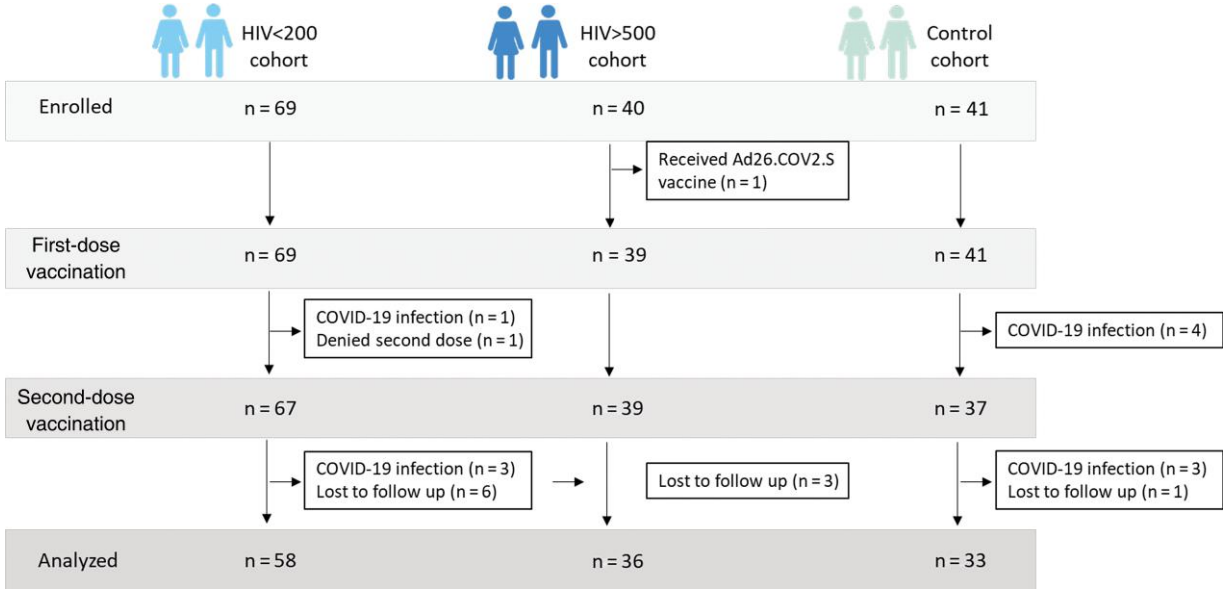

Figure 1. Flow diagram for the prospective observational cohort study according to the STROBE statement. Abbreviations: COVID-19, coronavirus disease 2019; HIV<200, people with human immunodeficiency virus (PWH) with CD4$^+$ T-cell counts <200/μL; HIV>500, PWH with CD4$^+$ T-cell counts >500/μL; STROBE, strengthening the reporting of observational studies in epidemiology (STROBE guideline).

in Vero E6 cells. Virus stock was prepared by collecting supernatant, sequenced as detailed elsewhere [14], and titrated in 10-fold serial dilutions to calculate the median tissue culture infective dose per milliliter. Virus was used at a multiplicity of infection of 7×10^{-4} to achieve a 50% cytopathic effect 3 days after infection.

Neutralization Assay

Neutralizing antibodies were measured in a randomly selected age-matched subgroup, representative of the total cohort, including 42 individuals from the HIV<200 group, 27 from the HIV>500 group , and 27 healthy controls. Neutralization assays were performed by preincubating 60 median tissue culture infectious dose (TCID50) of SARS-CoV-2 with 3-fold serial dilutions (1/60−1/14 580) of heat-inactivated plasma samples for 1 hour at 37°C. Preincubated viruses were added to 60 000 Vero E6 cells per well in 96-well plates. After 72 hours, the virus-induced cytopathic effect was measured using CellTiter-Glo Luminiscent cell viability reagent (Promega) and a Luminoskan Plate Reader (Thermo Fisher). Relative light units were normalized, and the half maximal inhibitory dilution (ID_{50}) (the dilution inhibiting 50% of the infection) was calculated by plotting and fitting plasma dilution log versus response to a 4-parameter equation, as described elsewhere [5,13,15].

Characterization of SARS-CoV-2–Specific T-Cell Responses by Enzyme-Linked Immunospot Assay

Cellular responses were analyzed by means of interferon (IFN) γ enzyme-linked immunospot (ELISPOT) assay in the 42 individuals from the HIV<200 group who were tested for neutralizing activity. After quality control, valid results were available from 30 of them. A peptide library of 425 peptides (15-18 aminoacid length, overlapping by 10 or 11 aminoacids, Synpeptide, China) was selected, as described elsewhere [16]; coverage is shown in Supplementary Table 1, and variants of concern (VOC) in Supplementary Table 2.

An IFN-γ ELISPOT kit (Mabtech) was used, according to the manufacturer's instructions. The peripheral blood mononuclear cells were thawed, rested for 4 hours at 37°C and 5% carbon dioxide, plated at $1.25–2 \times 10^5$ cells per well, stimulated with peptides (5 μg/mL), and cultured for 20 hours at 37°C and 5% carbon dioxide. Phytohemagglutinin (15 μg/mL; Sigma-Aldrich) was used as the positive control, and no stimuli as the negative control. Plates were revealed using BCIP/NBT-plus substrate solution (BioRad), and IFN-γ–secreting cells were quantified using an automated Cellular Technology Limited (C.T.L., OH, USA) ELISPOT Reader Unit [17]. The background response was defined as the mean number of spots for the negative control wells plus 3 times the standard deviation and was subtracted from the number from each well containing peptides.

Statistical Analysis

Sample size was based on the number of eligible adult PWH with CD4$^+$ T-cell counts <200/μL who agreed to participate. The differences between medians at baseline and at week 8 were compared using Wilcoxon matched-pairs signed rank tests, and Friedman tests were used to compare 3 time points. Differences between medians among groups were compared using Mann-Whitney or Kruskal-Wallis tests. Fisher exact tests were used to compare proportions among groups, and

Table 1. Baseline Characteristics of the Study Cohort at Inclusion

Characteristic	Value, Median (IQR)[a]			*P* Value[b]
	HIV<200 Group (n = 58)	HIV>500 Group (n = 36)	Control Group (n = 33)	
Age, y	52 (40–56)	51 (40–56)	53 (35–57)	.86[c]
Male sex, no. (%)	46 (79.3)	29 (80.6)	18 (54.5)	.02[d]
Route of HIV acquisition, no. (%)	…	…	…	.38
Sexual contact	39 (67.2)	25 (69.4)	NA	
IVDU	13 (22.4)	10 (27.8)	NA	
Other	6 (10.3)	1 (2.8)	NA	
pVL, $\log_{10}$ copies/mL	1.30 (1.30–1.52)	1.30 (1.30–1.30)	NA	
Undetectable pVL, no. (%)	47 (81)	36 (100)	NA	.006
Duration of virological suppression, y[e]	3.75 (1.09–14.88)	10.88 (6.63–11.42)	NA	.12
Time from HIV diagnosis to 1st COVID-19 vaccine, y	14.08 (1.69–28.31)	17.79 (3.50–28.32)	NA	.10
Absolute $CD4^+$ T-cell count, cells/μL	173 (117–257)	785 (655–966)	NA	<.001
CD4/CD8 ratio	0.23 (0.15–0.42)	1.21 (0.92–1.47)	NA	<.001
$CD4^+$ T-cell count nadir, cells/μL	64 (22–110)	259 (146–375)	NA	<.001

Abbreviations: COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; HIV<200, people with HIV with $CD4^+$ T-cell counts <200/μL; HIV>500, people with HIV with $CD4^+$ T-cell counts >500/μL; IQR, interquartile range; IVDU, intravenous drug user; NA, not applicable; pVL, plasma viral load.

[a]Data represent median (IQR) values except where otherwise specified.

[b]*P* values based on Mann-Whitney test except where otherwise specified.

[c]*P* value based on Kruskal-Wallis test.

[d]*P* value based on χ^2 test.

[e]Virological suppression was defined as a pVL <50 copies/mL. Only for individuals with pVL <50 at the time of inclusion.

Spearman rank tests were used for correlations. All tests were 2 sided, unadjusted for multiple comparisons, with $\alpha = 0.05$. GraphPad Prism software (version 9.1) was used for analysis and graphs.

RESULTS

Between 21 April and 5 July 2021, 58 individuals were included in the HIV<200 group, 36 in the HIV>500 group, and 33 in the HIV-1–negative control group. Nine individuals received BNT162b2, and 118 individuals were vaccinated with mRNA-1273. The demographics are summarized in Table 1. Participants were mainly male with a median (interquartile range [IQR]) age of 51 (35–57) years. The median (IQR) $CD4^+$ T-cell count and CD4/CD8 ratio at baseline were 173/μL (117–257/μL) and 0.23/μL (0.15–0.42/μL) in the HIV<200 group and 785/μL (655–966/μL) and 1.21/μL (0.92–1.47/μL) in the HIV>500 group, respectively ($P < .001$). In addition, the percentage of individuals with a plasma viral load <50 copies/mL within the year before enrollment was 81% in the HIV<200 and 100% in the HIV>500 group ($P = .006$).

Vaccine-Induced Humoral Immune Responses Against SARS-CoV-2

WWe assessed anti-S IgG and anti-RBD IgG at enrollment and at weeks 4 and 8 in all participants to identify potential differences among groups. All groups showed a significant increase in anti-S and anti-RBD IgG titers after the first and second doses (Figure 2; $P < .001$). However, anti-S and anti-RBD IgG titers in the HIV<200 group were significantly lower than in the HIV>500 and control groups, at weeks 4 and 8 (Figure 2*A* and 2*B*; $P < .05$). Specifically, the median (IQR) anti-S IgG titer at week 8 was 153.6 (26.4–654.9) binding antibody units (BAU)/mL in the HIV<200 group, 245.6 (145–824) BAU/mL in the HIV>500 group, and 274.7 (193.7–680.4) BAU/mL in the control group. Similarly, the median (IQR) anti-RBD IgG titers in the 3 groups were 171.9 (61.8–425.8), 555.8 (166.4–1751) and 281.6 (181–831.8) BAU/mL, respectively. Notably, there were no statistically significant differences between the HIV>500 and control groups (Figure 2).

Next, we classified all individuals into "low" and "high" anti-S IgG groups, according to whether the magnitude of S-specific IgG response at week 8 was either below or above 193.7 BAU/mL, a value that corresponds to the 25th percentile of the anti-S IgG levels seen in the control group. The proportion of PWH with low anti-S IgG levels was significantly higher than in the control group (Figure 2; $P = .008$). In terms of clinical variables, individuals with low anti-S IgG levels showed a trend towards being virologically suppressed for a shorter period than those with high levels (for 5 vs 11 years, respectively; $P = .052$; Table 2).

In a subgroup of individuals, we also evaluated the functional avidity of anti-S IgG and quantified the levels of anti-S IgM and IgA. We did not find any significant differences between groups ($P = .064$; Supplementary Figure 1), even after comparing individuals with low and high levels of anti-S IgG antibodies (Supplementary Figure 1). SARS-CoV-2 vaccination elicited similar anti-S IgA responses among all groups that were successfully boosted with each vaccine dose. However, individuals

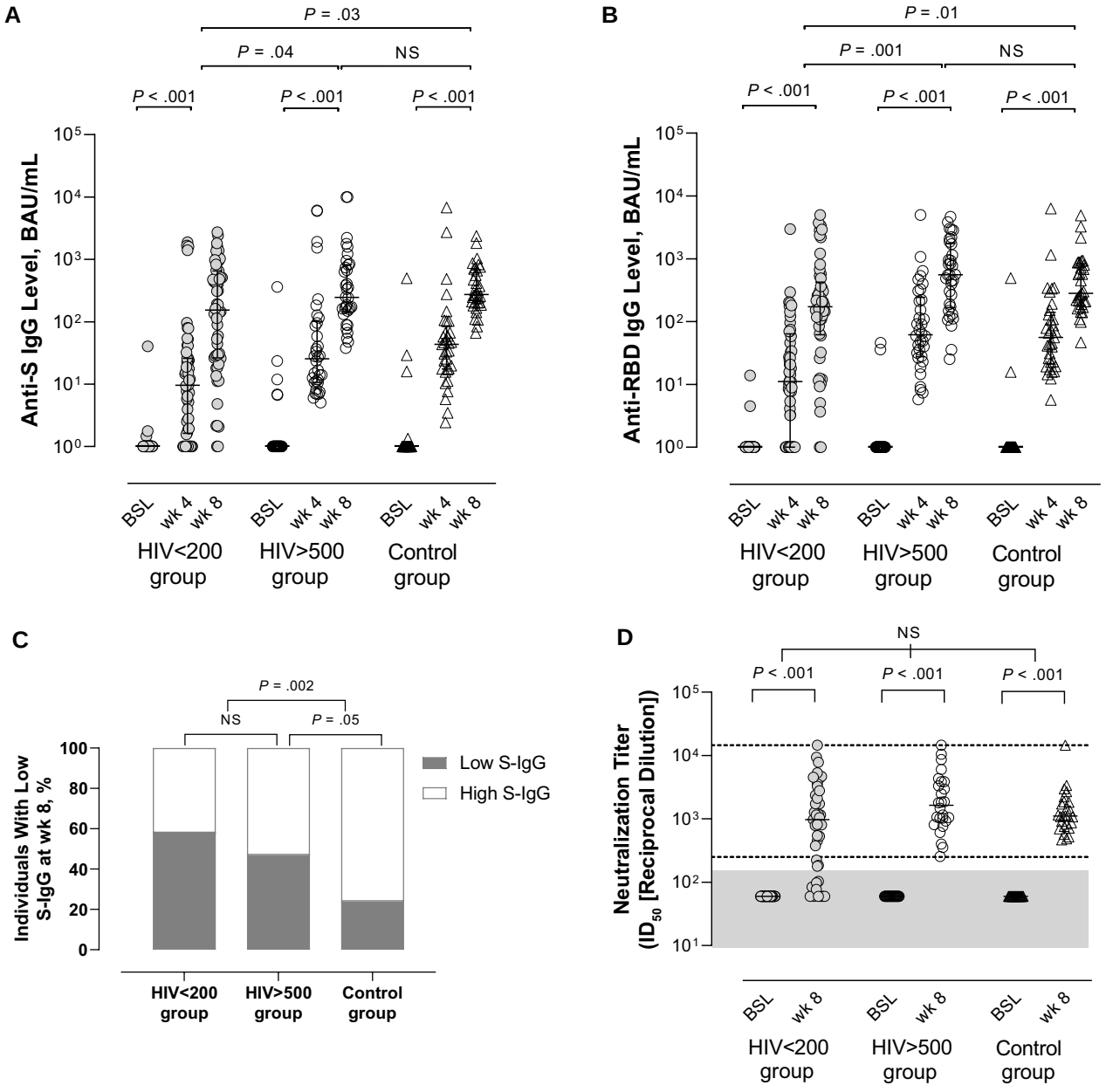

Figure 2. Vaccine-induced humoral immune responses in individuals from our cohort. *A, B,* Binding titers of immunoglobulin (Ig) antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S) (*A*) or receptor-binding domain (RBD) (*B*) in people with human immunodeficiency virus (PWH) with $CD4^+$ T-cell counts <200/μL (HIV<200 group), PWH with $CD4^+$ T-cell counts >500/μL (HIV>500 group), and the control group at baseline (BSL) and at weeks 4 and at 8 after vaccination. Abbreviation: NS, not significant. Antibody titers are displayed as medians with interquartile ranges (IQRs) and quantified in normalized binding antibody units (BAU). Friedman test was used to compare antibody titers at baseline and week 8 for each study group, and Mann-Whitney test to compare median levels of antibody at week 8 between groups. *C,* Proportion of individuals with "low" anti-S immunoglobulin (Ig) G levels (defined as <193.7 BAU/mL), according to the magnitude of anti-S IgG response at week 8. Fisher exact test is used for comparisons. *D,* SARS-CoV-2 neutralization titers (half maximal inhibitory dilution [ID_{50}], expressed as reciprocal dilution) from individuals in the HIV<200 group (n = 42), the HIV>500 group (n = 27), or the control group (n = 27) at baseline and at week 8. Median neutralization antibody titers and IQRs are depicted. Gray-shaded area shows titers <250, which are considered reduced neutralizing activity. Wilcoxon matched-pairs signed rank test was used to compare neutralization titers at BSL and week 8 for each study group, and Mann-Whitney test was used to compare median levels at week 8 between groups. All undetectable levels are represented as 1.

in the HIV<200 group showed higher IgM responses at week 8 than those in the HIV>500 and control groups (Supplementary Figure 2).

Overall, these results indicate that PWH are able to elicit immunogen-specific humoral immune responses after 2 doses of mRNA vaccination, even though the magnitude of this response

Table 2. Baseline Characteristics of People With Human Immunodeficiency Virus With Low or High Immunoglobulin G Antibody Levels Against Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein

Characteristic	Value, Median (IQR)[a]		
	Low Anti-S IgG (n = 50)	High Anti-S IgG (n = 43)	*P* Value[b]
Age, y	51 (40–57)	52 (43–56)	.75
Undetectable pVL, no. (%)	42 (84)	40 (93)	.21[c]
Duration of virological suppression, y[d]	5 (1–22)	11 (3–13)	.05
Time from HIV diagnosis to 1st COVID-19 vaccine, y	14 (3–29)	19 (7–29)	.33
Absolute $CD4^+$ T-cell count, cells/μL	254 (132–663)	346 (171–780)	.19
CD4/CD8 ratio	0.83 (0.21–1.44)	0.69 (0.25–1.4)	.63
$CD4^+$ T-cell count nadir, cells/μL	101 (60–553)	115 (23–257)	.71

Abbreviations: Anti-S IgG, immunoglobulin G antibody against severe acute respiratory syndrome coronavirus 2 spike protein; COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; IQR, interquartile range; pVL, plasma viral load.

[a]Data represent median (IQR) values except where otherwise specified.

[b]*P* values based on Mann-Whitney test except where otherwise specified.

[c]*P* value based on Fisher exact test.

[d]Virological suppression was defined as a pVL <50 copies/mL.

is significantly weaker in PWH with $CD4^+$ T-cell counts < 200/μL than in those with counts >500/μL.

Vaccine-Induced Neutralizing Activity Against SARS-CoV-2

To determine whether SARS-CoV-2–specific antibodies generated after vaccination were able to block the virus, we tested the neutralization capacity of the plasma in a subgroup of individuals at baseline and at week 8, using an assay based on wild-type SARS-CoV-2 [5]. At baseline, 5 participants already had detectable neutralizing activity (Figure 2), most likely owing to previous asymptomatic SARS-CoV-2 infection, but they were not excluded from the analysis. There was a significant increase in neutralization titers from baseline to week 8 in all groups ($P < .001$), showing similar neutralization levels (Figure 2). No clear cutoff for a neutralizing activity that unambiguously protects against SARS-CoV-2 infection has been established. Nevertheless, based on data gathered from an outbreak, neutralizing activities above 1:250 is a cutoff that might be strong enough to prevent infection [13,18]. We identified 33.3% individuals in the HIV<200 group (n = 13), compared with 3.7% in the HIV>500 (n = 1) and none in the control group, showing an ID_{50} of <1:250 (referred to as "low neutralizers"; $P < .001$; Figure 2).

Levels of neutralizing activity were positively correlated with anti-S and anti-RBD antibody titers in all groups at week 8 ($P < .001$; Spearman correlation, $\rho = 0.7715$ and $\rho = 0.6276$, respectively; Figure 3). Given that 19% of individuals from the HIV<200 group had detectable viremia at inclusion, we analyzed the two groups (HIV<200/pVL>50 and HIV<200/pVL<50) separately to see if the impaired immune responses observed could be driven by the viremic grou. We did not find statistically significant differences between groups for each of the covariates of interest (anti-S and anti-RBD IgG titers at baseline, w4 and w8 and anti-ID_{50} at baseline and at w8) (all p-values >.05 for Mann-Whitney test). Overall, these data suggest that, in the context of PWH with poor immune reconstitution, 1 in 3 individuals showed low levels of neutralizing antibodies that might be below the protective cutoff against SARS-CoV-2 after 2 doses of mRNA vaccine.

Vaccine-Induced Cellular Immune Responses Against SARS-CoV-2

To assess whether PWH with $CD4^+$ T-cell counts <200/μL were able to mount SARS-CoV-2–specific T-cell responses despite their impaired humoral responses after vaccination, we used ELISPOT assays to measure IFN-γ–secreting cells in a subgroup of individuals selected according to their neutralization activity. "High neutralizers" showed a tendency toward an increase in SARS-CoV-2–specific T-cell responses directed against the S1 subunit (>4-fold increase; $P = .07$) from baseline to week 8.

Notably, we identified significant differences in peptide pools B50–B51, corresponding to the C-half of the S1 subunit, and B53–B54, which covers part of RBD region (Figure 4). However, we did not observe significant increases in cellular responses against the S2 subunit (Figure 4; $P = .39$), even after exclusion of the B82 and B83 peptide pools, which cover the fusion peptide and are the most cross-reactive regions to other human coronaviruses [19–21]. By contrast, low neutralizers were unable to induce SARS-CoV-2–specific T-cell responses to any specific pool of peptides tested for the S1 and S2 subunits (Figure 4). Thus, the overall magnitude of cellular responses against S and the RBD regions at week 8 were significantly higher in high neutralizers than in low neutralizers (Figure 5; $P = .02$; 2- vs 0.7-fold increase, respectively).

Regarding SARS-CoV-2 specific T-cell responses against VOC at week 8, we observed a significant loss of IFN-γ–secreting cells against the Alpha ($P = .001$) and Kappa, Delta, and B.1.617.3 VOC (all $P = .01$) in high neutralizers (Supplementary Figure 3). Taken together, these data suggest a lack of vaccine-induced T-cell immunity against SARS-CoV-2 after 2 doses of mRNA vaccine in PWH with $CD4^+$ T-cell counts <200/μL.

DISCUSSION

We conducted a comprehensive analysis of a large cohort of PWH with poor immune reconstitution. Here, beyond anti-S IgG measurements already reported [22–25], we analyzed functional avidity of IgG, neutralization capacity using replicative SARS-CoV-2, and the cellular immunity of these individuals. Overall, these data reveal a complete picture of this high-risk population, in which approximately one-third of individuals

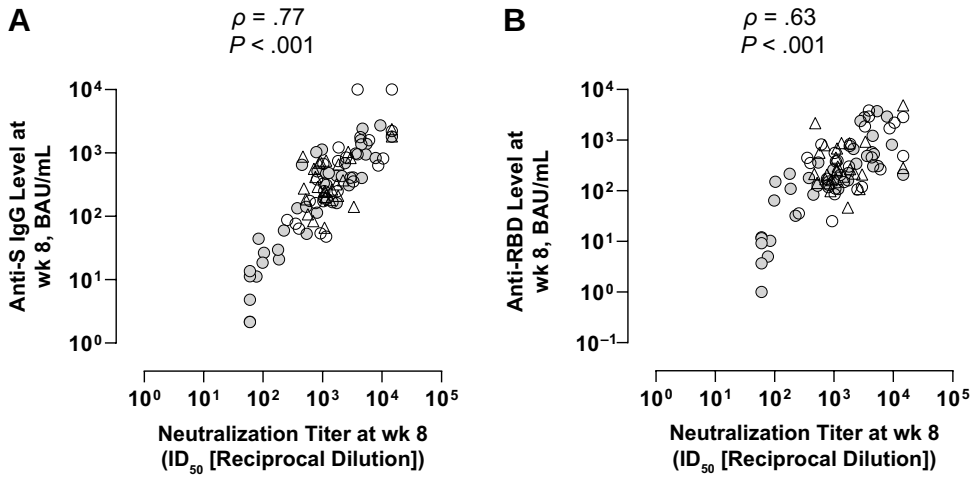

Figure 3. Association between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralization titers and levels of binding antibodies at week 8. *A,* Association between SARS-CoV-2 neutralization titers and levels of binding anti–spike protein (S) immunoglobulin (Ig) G antibodies against the virus from individuals analyzed at week 8. Spearman correlation was used. *B,* Association between SARS-CoV-2 neutralization titers and levels of binding anti–receptor-binding domain (RBD) IgG antibodies against the virus from individuals analyzed at week 8. Spearman correlation was used. All undetectable levels are represented as 1. Abbreviations: BAU, binding antibody units; ID_{50}, half maximal inhibitory dilution.

have low IgG levels, reduced in vitro neutralization activity, and no vaccine-induced T cells 4 weeks after the second mRNA vaccine dose.

Impaired immune responses to pneumococcus [26], influenza [27], and hepatitis A and B [28] are widely described in PWH. Previous studies have shown that primary responses and the maintenance of long-term serological memory are affected during HIV-1 infection [29,30]. The inability of $CD4^+$ T cells to effectively activate B cells [31] and the HIV-associated premature exhaustion of B cells could cause a suboptimal humoral response to vaccination [32]. Importantly, vaccine responsiveness remains often diminished despite optimal ART suppression [26].

In our cohort, 20% of individuals in the HIV<200 group had detectable HIV-1 viraemia. Chronic immune activation/exhaustion associated with incompletely HIV suppression could impact vaccine-induced humoral responses as seen for other vaccinations [26]. However, our findings suggest that the impaired immune responses observed in the HIV<200 group are not only driven by the viremic group. These findings further support applied recommendations on priority access to SARS-CoV-2 vaccination in these individuals regardless off their pVL rather than delaying vaccination until ART-suppression was achieved. In contrast to S-specific IgG, the role of anti-S IgM and IgA is less well characterized. Interestingly, it has been shown that the coexistence of anti-S IgG and IgM was associated with improved anti–SARS-CoV-2 humoral response and with higher neutralizing activity in plasma [33]. It has been documented that after natural infection, IgA dominates the early neutralizing humoral response to SARS-CoV-2 [34]. In addition, mRNA SARS-CoV-2 vaccination successfully boosted mucosal IgA response in convalescent individuals [35]. In the current study, individuals in the HIV<200 group showed comparable IgA titers but higher IgM responses than those in the HIV>500 and control groups. This increase of anti-S IgM responses could be a consequence of differences in their B-cell repertoire 44 and higher basal inflammation as it has been previously described in other infections [36,37].

In the current study, using a virus isolated during the first COVID-19 wave in Spain in March 2020 and harboring the S D614G mutation, 33% of individuals in the HIV<200 group showed reduced levels of neutralization at week 8 after vaccination. These results confirm the findings reported in an Italian cohort of PWH with different degrees of immune recovery [38]. Thus, it is likely that in our cohort, an even higher proportion of immunosuppressed individuals would have showed reduced neutralizing activity in vivo against more divergent and contemporaneous VOC, such as the Beta, Delta, and Omicron variants.

In addition to the role of neutralizing antibodies in SARS-CoV-2 viral control, there is growing evidence for an important contribution of virus-specific T-cell responses to limit disease progression towards severe COVID-19 [19,39–42]. We showed here an impaired vaccine-induced T-cell immunity against SARS-CoV-2 in PWH who had low levels of anti-S IgG and were low neutralizers. These results are further supported by a study showing that the magnitude of SARS-CoV-2–specific T-cell responses after natural infection in PWH is positively correlated with naive $CD4^+$ T-cell counts and the CD4/CD8 ratio [43]. These findings could be explained in part by the reduced production of interleukin 2 from antigen-specific $CD8^+$ T cells in PWH, which could hinder their proliferative

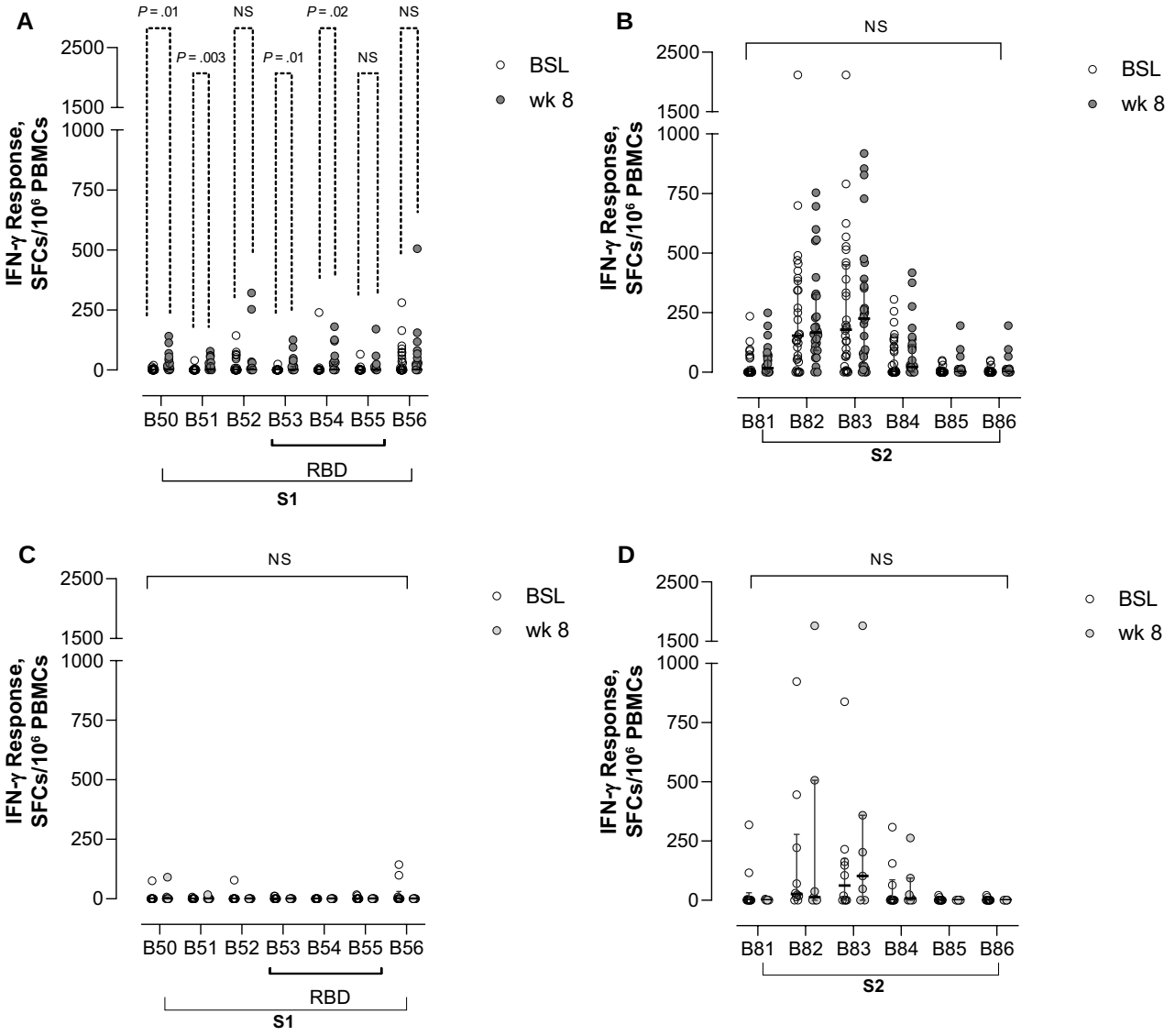

Figure 4. Vaccine-induced T-cell immune responses against the S1 and S2 subunits of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S) in "high neutralizers" and "low neutralizers" among people with human immunodeficiency virus with CD4$^+$ T-cell counts <200/μL (HIV<200 group). *A, B,* Magnitude of interferon (IFN) γ response, in spot-forming cells (SFCs) per million peripheral blood mononuclear cells (PBMCs), for SARS-CoV-2 peptide pools covering S1 (*A*) and S2 (*B*) subunits in high neutralizers at baseline (BSL) and at week 8 after vaccination. Abbreviations: NS, not significant; RBD, receptor-binding domain. *C, D,* Magnitude of IFN-γ response for SARS-CoV-2 peptide pools covering S1 (*C*) and S2 subunits (*D*) in low neutralizers at baseline and at week 8 after vaccination. Wilcoxon matched-pairs signed rank test is used, not adjusted for multiple comparisons. Median and interquartile range is shown.

potential and long-term immune memory following natural infection and/or immunization [44]. Importantly, high neutralizers showed reduced T-cell responses against some VOC, which probably can be more relevant with contemporaneous VOC, such as Delta and Omicron. Our findings demonstrated substantial background in the S2 subunit when compared SARS-CoV-2 basal and post-vaccinated individuals. This is in line with other studies that found S reactive CD4+ T-cells to epitopes at the C-terminal of the S region [42] and the strongest IFN-γ in region outside the RBD [45]. Indeed, the S2 subunit displays homology with human endemic coronaviruses, such as NL63, 229E, HKU1 and OC43 [42,46,47] and could develop cross-reactive responses in uninfected individuals.

Given the impaired humoral and cellular immune responses after 2 SARS-CoV-2 vaccine doses in a significant proportion of PWH with CD4$^+$ T-cell counts <200/μL, our data suggest that these individuals could benefit from close monitoring to prioritize them for alternative strategies aimed to achieve better immunity against SARS-CoV-2 but especially to limit severe disease outcomes. These strategies could consist of additional doses (homologous or heterologous boosters) together with a close monitoring of antibody levels to detect suboptimal

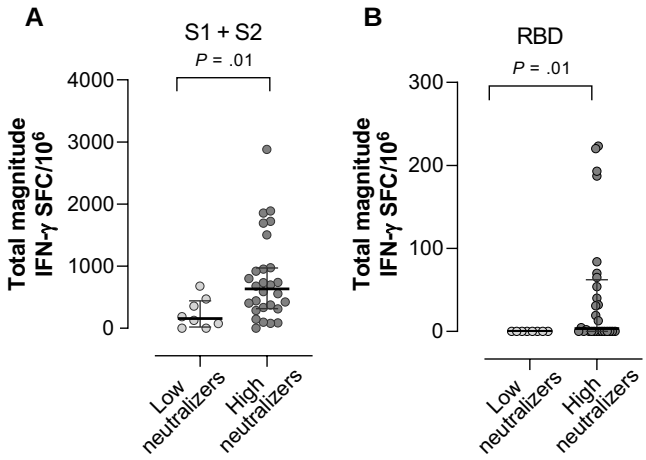

Figure 5. Comparison of vaccine-induced T-cell responses at week 8 between "high neutralizers" and "low neutralizers" among people with human immunodeficiency virus with $CD4^+$ T-cell counts <200/μL (HIV<200 group). Total magnitude of interferon (IFN) γ response, in spot-forming cells (SFCs) per million peripheral blood mononuclear cells (PBMCs), for spike protein (S) (*A*) and receptor-binding domain (RBD) peptide pools (*B*) in low and high neutralizers at week 8 after vaccination. Scatterplots show medians with interquartile ranges. We used the Mann-Whitney test in unpaired samples and the Wilcoxon test in paired samples for statistical analysis.

responsiveness, such as what is contemplated in the ongoing European Union–funded project RBDCOV (101046118). Because we identified a positive correlation between neutralization activity and anti-S IgG levels, these individuals might be easily identified using quick antibody detection methods.

Although vaccination remains the most important intervention available to lower the risk of severe disease, the use of neutralizing monoclonal antibodies—such as the combination of tixagevimab and cilgavimab (Evusheld)—to prevent SARS-CoV-2 acquisition [48] suggests that passive antibody prophylaxis could be an approach to consider in PWH who do not have an adequate immune response to vaccination. Sincewe showed that PWH with $CD4^+$ T-cell counts <200/μL have impaired vaccine responsiveness, which is in line with previous findings [38], the use of Evusheld or other future antibodies less resistant to contemporaneous variants in this population, warrants consideration.

The current study has some limitations. First, we did not assess immune responses in PWH with $CD4^+$ T-cell counts between 200/μL and 500/μL. Second, the latest time point analyzed in our study was 1 month after the second vaccine dose. Importantly S-IgG levels have been shown to wane fast and booster vaccinations have been progressively recommended for everyone. Moreover, we did not performed anti-N antibodies to exclude individuals with asymptomatic infection from the analysis, but we still were able to detect one third o individuals with CD4+ T-cell counts <200/μL with impaired immune responses after vaccination. Finally, other functions of antibodies such as antibody-dependent cellular cytotoxicity that might also impact COVID-19 severityor activity against more contemporaneous VOC, has not been assessed.

In conclusion, our study demonstrated that 1 in 3 PWH with $CD4^+$ T-cell counts <200/μL reached low levels of anti-S and anti-RBD IgG, together with weak neutralization activity and absence of cellular responses. These individuals would benefit from monitoring vaccine responsiveness and support their prioritization for additional booster vaccinations and/or alternative preventive approaches against SARS-CoV-2.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med **2021**; 384:403–16.
2. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet **2021**; 397:99–111.
3. Gilbert PB, Montefiori DC, McDermott A, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy trial. medRxiv [Preprint: not peer reviewed]. Posted 15 August 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.08.09.21261290v4.
4. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med **2021**; 27:1205–11.
5. Trinité B, Tarrés-Freixas F, Rodon J, et al. SARS-CoV-2 infection elicits a rapid neutralizing antibody response that correlates with disease severity. Sci Rep **2021**; 11:1–10.
6. Geretti AM, Stockdale AJ, Kelly SH, et al. Outcomes of coronavirus disease 2019 (COVID-19) related hospitalization among people with human immunodeficiency virus (HIV) in the ISARIC World Health Organization (WHO) clinical characterization protocol (UK): a prospective observational study. Clin Infect Dis **2021**; 73:e2095–106.
7. Vizcarra P, Pérez-Elías MJ, Quereda C, et al; on behalf of the COVID-19 IT. Description of COVID-19 in HIV-infected individuals: a single-centre, prospective cohort. Lancet HIV **2020**; 7:e554–64.
8. Dandachi D, Geiger G, Montgomery MW, et al. Characteristics, comorbidities, and outcomes in a multicenter registry of patients with human immunodeficiency virus and coronavirus disease 2019. Clin Infect Dis **2021**; 73:e1964–72.
9. Madhi SA, Koen AL, Izu A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in people living with and without HIV in South Africa: an interim analysis of a randomised, double-blind, placebo-controlled, phase 1B/2A trial. Lancet HIV **2021**; 8:e568–80.
10. Levy I, Wieder-Finesod A, Litchevsky V, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in people living with HIV-1. Clin Microbiol Infect **2021**; 27:1851–5.
11. Woldemeskel BA, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA vaccine elicits robust humoral and cellular immune responses in people living with human immunodeficiency virus (HIV). Clin Infect Dis **2022**; 74:1268–70.
12. Frater J, Ewer KJ, Ogbe A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. Lancet HIV **2021**; 8:e474–85.
13. Pradenas E, Trinité B, Urrea V, et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. Med **2021**; 2:313–20.e4.
14. Rodon J, Muñoz-Basagoiti J, Perez-Zsolt D, et al. Identification of plitidepsin as potent inhibitor of SARS-CoV-2-induced cytopathic effect after a drug repurposing screen. Front Pharmacol **2021**; 12:1–12.
15. Trinité B, Pradenas E, Marfil S, et al. Previous SARS-CoV-2 infection increases b.1.1.7 cross-neutralization by vaccinated individuals. Viruses **2021**; 13:1135.
16. Olvera A, Noguera-Julian M, Kilpelainen A, Romero-Martín L, Prado JG, Brander C. SARS-CoV-2 consensus-sequence and matching overlapping peptides design for COVID19 immune studies and vaccine development. Vaccines **2020**; 8:1–14.
17. Dalmau J, Rotger M, Erkizia I, et al. Highly pathogenic adapted HIV-1 strains limit host immunity and dictate rapid disease progression. AIDS **2014**; 28:1261–72.
18. Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol **2020**; 58:e02107-20.
19. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell **2020**; 183:158–68.e14.
20. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature **2020**; 584:457–62.

21. Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature **2020**; 587:270–4.
22. Nault L, Marchitto L, Goyette G, et al. Covid-19 vaccine immunogenicity in people living with HIV-1. Vaccine **2022**; 40:3633–7.
23. Hassold N, Brichler S, Ouedraogo E, et al. Impaired antibody response to COVID-19 vaccination in advanced HIV infection. AIDS **2022**; 36:F1–5.
24. Noe S, Ochana N, Wiese C, et al. Humoral response to SARS-CoV-2 vaccines in people living with HIV. Infection **2021**; 50:617–23.
25. Corma-Gómez A, Fernández-Fuertes M, García E, et al. Severe immunosuppression is related to poorer immunogenicity to SARS-CoV-2 vaccines among people living with HIV. Clin Microbiol Infect **2022**.
26. Zhang L, Li Z, Wan Z, Andrew Kilby JMK. Humoral immune responses to *Streptococcus pneumoniae* in the setting of HIV-1 infection. Vaccine **2015**; 33:4430–6.
27. Xia Y, Mi F, Du G, Qin S. Analysis of protective immune responses to seasonal influenza vaccination in HIV-infected individuals. Hum Vaccines Immunother **2021**; 17:124–32.
28. Mena G, García-Basteiro AL, Bayas JM. Hepatitis B and A vaccination in HIV-infected adults: a review. Hum Vaccines Immunother **2015**; 11:2582–98.
29. Kernéis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boëlle PY. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. Clin Infect Dis **2014**; 58:1130–9.
30. Titanji K, De Milito A, Cagigi A, et al. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood **2006**; 108:1580–7.
31. Goncalves L, Albarran B, Salmen S, et al. The nonresponse to hepatitis B vaccination is associated with impaired lymphocyte activation. Virology **2004**; 326:20–8.
32. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med **2008**; 205:1797–805.
33. Ruggiero A, Piubelli C, Calciano L, et al. SARS-CoV-2 vaccination elicits unconventional IgM specific responses in naïve and previously COVID-19-infected individuals. eBioMedicine **2022**; 77:103888.
34. Sterlin D, Malaussena A, Gorochov G. IgA dominates the early neutralizing antibody response to SARS-CoV-2 virus. Med Sci (Paris) **2021**; 37:968–70.
35. Chan RWY, Liu S, Cheung JY, et al. The mucosal and serological immune responses to the novel coronavirus (SARS-CoV-2) vaccines. Front Immunol **2021**; 12:1–9.
36. Carrillo J, Negredo E, Puig J, et al. Memory B cell dysregulation in HIV-1-infected individuals. AIDS **2018**; 32(2): 149–160.
37. Hel Z, Xu J, Denning WL, et al. Dysregulation of systemic and mucosal humoral responses to microbial and food antigens as a factor contributing to microbial translocation and chronic inflammation in HIV-1 infection. PLOS Pathog **2017**; 13:e1006087.
38. Antinori A, Cicalini S, Meschi S, et al. Humoral and cellular immune response elicited by mRNA vaccination against SARS-CoV-2 in people living with HIV (PLWH) receiving antiretroviral therapy (ART) according with current CD4 T-lymphocyte count. Clin Infect Dis **2022**; 75:e552–63.
39. Ju B, Zhang Q, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature **2020**; 584:115–9.
40. Seow J, Graham C, Merrick B, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. Nat Microbiol **2020**; 5:1598–607.
41. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell **2020**; 181:1489–501.e15.
42. Peng Y, Mentzer AJ, Liu G, et al. Broad and strong memory $CD4^+$ and $CD8^+$ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol **2021**; 21:1336–45.
43. Alrubayyi A, Gea-Mallorquí E, Touizer E, et al. Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with HIV. Nat Commun **2021**; 12:1–16.
44. Zimmerli SC, Harari A, Cellerai C, Vallelian F, Bart P, Pantaleo G. HIV-1-specific IFN-gamma/IL-2-secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells. Proc Natl Acad Sci **2005**; 102:7239–44.
45. Nelde A, Bilich T, Heitmann JS, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. Nature Immunol **2021**; 22:74–85.
46. Tarke A, Sidney J, Kidd CK, et al. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. Cell Reports Medicine **2021**; 2:100204.
47. Zhao J, Wang L, Schank M. SARS-CoV-2 specific memory T cell epitopes identified in COVID-19-recovered subjects. Virus Res **2021**; 304:198508.
48. Levin MJ, Ustianowski A, De Wit S, et al. Intramuscular AZD7442 (Tixagevimab–Cilgavimab) for Prevention of Covid-19. N Engl J Med **2022**; 386:2188–2200.