Limited humoral and specific T-cell responses after SARS-CoV-2 vaccination in PLWH with poor immune reconstitution

Running title:
COVID-19 vaccines in PLWH

Susana Benet1,*, Oscar Blanch-Lombarte2,*, Erola Ainsua-Enrich2,*, Nuria Pedreño-Lopez2,*, Jordana Muñoz-Basagoiti2,#, Dàlia Raich-Regue2,#, Daniel Perez-Zsolt2,#, Ruth Peña2, Esther Jiménez2, María Luísa Rodríguez de la Concepción2, Carlos Ávila2, Samandhy Cedeño2, Tuixent Escribà2, Luis Romero-Martín2, Yovanimma Alarcón-Soto1, Gabriel Felipe Rodríguez-Lozano2, Cristina Miranda1, Sandra González1, Lucía Bailón1,8, Julià Blanco2,3,4,5, Marta Massanella2,5, Christian Brander2,4,5,6, Bonaventura Clotet1,2,3,4, Roger Paredes1,2,3,4,5, María Esteve7,8, Nuria Izquierdo-Useros2,3,5, Jorge Carrillo2,3,5, Julia G. Prado2,3,5, José Moltó1,5,¥ and Beatriz Mothe1,2,4,5,¥.

Affiliations:
1Fundació llibert contra la sida, Infectious Diseases Department, Hospital Germans Trias I Pujol, 08916, Badalona, Spain
2IrsiCaixa AIDS Research Institute, Hospital Germans Trias I Pujol, 08916, Badalona, Spain
3Germans Trias i Pujol Research Institute (IGTP), 08916, Badalona, Spain
4Faculty of Medicine, University of Vic-Central University of Catalonia (UVic-UCC), Vic, Spain.
ABSTRACT

Background. We analyzed humoral and cellular immune responses induced by SARS-CoV-2 mRNA vaccines in people living with HIV-1 (PLWH) with < 200 CD4+ T-cells.

Methods. Prospective cohort study including 58 PLWH with CD4+ T-cell counts <200 cells/mm³, 36 with CD4+ T-cell counts >500, and 33 HIV-1-negative controls. Antibodies against the SARS-CoV-2 Spike protein (anti-S IgG) and the receptor-binding domain (anti-RBD IgG) were quantified before and four weeks after the first and the second dose of BNT162b2 or mRNA-1273 (w8). Viral neutralization activity and T-cell responses were also determined.

Results. At w8, anti-S/anti-RBD IgG responses increased in all groups (P<0.0001). Median (IQR) S-IgG and RBD-IgG at w8 were 153.6 (26.4; 654.9) and 171.9 (61.8; 425.8) in the HIV<200 group compared to 245.6 (145; 824) and 555.8 (166.4; 1751) in the HIV>500 group, and 274.7 (193.7; 680.4) and 281.6 (181; 831.8) BAU/mL in controls (P<0.05). Neutralizing capacity and specific T-cell immune responses were absent or reduced in 33% of the HIV<200 group, compared with 3.7% in the HIV>500 (P=0.0003).

Conclusion. One third of PLWH with CD4+ T-cell counts <200 cells/mm³ 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 COVID-19 mRNA vaccines.

Keywords:
SARS-CoV-2 vaccine, people living with HIV (PLWH), anti-S IgG, anti-RBD IgG, neutralizing antibodies
INTRODUCTION

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 health care system. Since the first case reported in Wuhan, China, close to 480 million cases have been documented worldwide, causing over 6 million deaths as of March 2022 (https://covid19.who.int/).

Successful development of safe and effective vaccines has drastically reduced morbidity and mortality associated with COVID-19[1,2]. Both, messenger RNA (mRNA) and adenovirus-vectored vaccines show high effectiveness at 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) correlate with vaccine efficacy[3–5].

People living with HIV (PLWH) are at higher risk to develop severe COVID-19 disease[6,7], especially those individuals aging 40 years or older, those with low CD4+ T-cell counts (<200 cells/mm³) and/or those with three or more comorbidities[8]. Consequently, PLWH with low CD4+ T-cell counts have received priority access to SARS-CoV-2 vaccines in many vaccine roll-out programs. However, this population was either excluded or underrepresented in pivotal phase 2/3 vaccine trials as these studies only allowed inclusion of PLWH with high CD4+ T-cell counts on suppressive ART[1]. While PLWH with well-controlled infection showed similar humoral responses compared to general population[9–12], data on vaccine-induced responses from PLWH at higher risk of severe COVID-19 disease are still scarce. This information is essential for the clinical management of this group patients.
Our objective was to characterize humoral and cellular immune response after SARS-CoV-2 vaccination in PLWH with low CD4+ T-cell counts and/or with suboptimal viral suppression.

**MATERIAL AND METHODS**

**Study design**

Prospective observational single-center cohort study to investigate vaccine-specific immune responses after 2 doses of mRNA-based SARS-CoV-2 vaccination in PLWH with distinct levels of immune status compared to aged-matched HIV-negative controls. The primary endpoint was to assess antibody levels against S and RBD (anti-S and anti-RBD IgG) after primary (2-dose) vaccination. Antibody levels were measured in all participants at baseline, 21 or 28 days after the first vaccination, and at 28 days after the second vaccination (named w4 and w8 for consistency). Secondary endpoints included determination of avidity, IgM/IgA measurements, neutralization activity and vaccine-induced cellular immune responses, which were assessed at baseline and at w8 in a subgroup of clinically representative individuals of the total cohort based on sample availability.

All participants included in the study received two doses of mRNA SARS-CoV-2 vaccines (BNT162b2 -21 days apart- or mRNA-1273 -28 days apart-) following the National Vaccination Plan for primary doses. Peripheral blood mononuclear cells (PBMCs) and plasma samples were collected at baseline, at w4 and w8. Demographic data were collected from electronic medical records.
The study was approved by the Institutional Ethical Review Board at Hospital Universitari Germans Trias I Pujol (HUGTiP) in Badalona, Spain (PI-21-108). All participants gave written informed consent before enrollment. Biological biosafety committee approved SARS-CoV-2 experiments at the BSL3 laboratory of the Center for Bioimaging and Comparative Medicine (CSB-20-015-M3).

**Study population**

In April 2021, once vaccine-rollout prioritized PLWH with CD4⁺ T-cell counts <200 cells/mm³, we searched for individuals aging >18 years with <200 CD4⁺ T-cells/mm³ within the last year out of PLWH under regular care at HUGTiP. Exclusion criteria included previous SARS-CoV-2 vaccination and known history of SARS-CoV-2 infection (Fig.1, Flow diagram)

PLWH with >500 CD4⁺ T-cell counts and HIV-1 negative controls, mostly represented by healthcare workers, were included sequentially through opportunistic vaccination at HUGTiP and were matched by age with HIV<200 group.

**Enzyme-linked immunosorbent assay**

We measured anti-S IgG and anti-RBD IgG antibody levels at enrollment, and at w4 and w8 in 127 participants who completed follow-up, except for one individual in the HIV<200 group, whose results were not valid, and S-IgG avidity and anti-S IgM and IgA antibodies in 52 randomly-selected participants (n=26 in HIV<200 group, n=13 in HIV>500 group and n=13 in control group). Plates were coated overnight at 4°C with the HIS.H8 antibody (ThermoFisher Scientific) at 2µg/mL, blocked with PBS+1% bovine serum albumin (BSA, Miltenyi biotech) for two hours at room temperature (RT), washed and incubated with: S, RBD or Nucleocapsid protein (all from Sino Biological)
at 1µg/ml or PBS/1%BSA to estimate sample background. A positive plasma sample was used as standard, and a pool of 10 SARS-CoV-2 negative plasma samples served as negative control. Samples were incubated for one hour (RT). Secondary antibodies: HRP 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 (OPD) (Sigma Aldrich) was added and the enzymatic reaction was stopped with 2M of H₂SO₄ (Sigma Aldrich). Signal was evaluated as the optical density (OD) at 492 nm with noise correction at 620 nm. Specific signal for each antigen was determined after subtracting background obtained from antigen-free wells. Results are expressed as arbitrary units (AU/mL) according to the standard[13]

S-IgG avidity was evaluated by ELISA. Plates were coated with S (Sino Biological) at 1µg/ml and were blocked with PBS/1%BSA (Miltenyi biotech) for two hours (RT). Samples were diluted at 0.5 AU/mL and evaluated in quadruplicate for one hour (RT). After washing, 2M Guanidine-HCl or PBS were added and incubated for 15 minutes (RT). Bound antibodies were detected using HRP conjugated goat anti-human IgG (Jackson ImmunoResearch). Plates were revealed using OPD (Sigma Aldrich). Enzymatic/reaction was stopped with 2M of H₂SO₄ (Sigma Aldrich). Signal was evaluated as OD at 492 nm with noise correction at 620 nm. S-IgG avidity was calculated as the ratio between mean signal obtained with and without guanidine treatment.
Viral isolation and titration
SARS-CoV-2 was isolated from a nasopharyngeal swab collected in March 2020 in Spain and propagated for two passages in Vero E6 cells. Virus stock was prepared by collecting supernatant, sequenced as detailed previously[14] and titrated in 10-fold serial dilutions to calculate the TCID$_{50}$/mL. Virus was used at an MOI of $7 \cdot 10^{-4}$ to achieve 50% cytopathic effect 3 days post-infection.

Neutralization Assay
Neutralizing antibodies were measured in a randomly selected subgroup, representative of the total cohort, of 42 individuals from HIV<200 group, 27 individuals from HIV>500 group and 27 from control group, which were matched by age with HIV<200 group.
Neutralization assays were performed preincubating 60 TCID50 of SARS-CoV-2 with three-fold serial dilutions (1/60 to 1/14580) of heat-inactivated plasma samples for 1h at 37 ºC. Pre-incubated viruses were added to 60.000 Vero E6 cells per well in 96-well plates. 72 hours later, viral-induced cytopathic effect was measured using CellTiter-Glo Luminiscent cell viability reagent (Promega) and Luminoskan Plate Reader (Thermofisher). Relative light units (RLU) were normalized, and ID$_{50}$ (the dilution inhibiting 50% of the infection) was calculated by plotting and fitting plasma dilution log vs. response to a 4-parameter equation as previously described[5,13,15].

Characterization of SARS-CoV-2-specific T-cell responses by ELISpot
Cellular responses were analyzed by IFN-γ ELISpot in those 42 individuals from the HIV<200 group who were tested for neutralizing activity. After quality control, valid results were available from 30 individuals.
A peptide library of 425 15-18mers (overlapping by 10 or 11 amino acids, Synpeptide, China) was selected as previously described[16], coverage shown in Supplementary Table 1, and variants of concern (VOC) described in Supplementary Table 2.

IFNγ ELISpot kit (Mabtech) was used following manufacture instructions. PBMCs were thawed, rested for 4 hours at 37°C and 5% of CO₂, plated at 1.25 – 2·10⁵ cells/well, stimulated with peptides (5 µg/ml), and cultured for 20 hours at 37°C and 5% of CO₂. PHA (15 µg/ml, Sigma-Aldrich) was used as positive control, and no stimuli as negative control. Plates were revealed using BCIP/NBT-plus substrate solution (BioRad) and IFNγ-secreting cells were quantified under ImmunoCapture and ImmunoSpot software[17]. Background responses were defined as mean number of spots of the negative control wells plus three times the standard deviation, and subtracted from each well containing peptides.

**Statistical analysis**

Sample size was based on the eligible number of adult PLWH with <200 CD4⁺ T-cells/mm³ who accepted to participate. Differences of medians between baseline and w8 were compared using Wilcoxon matched-pairs signed rank test and for comparisons between 3 timepoints Friedman test was used. Differences of medians among groups were compared using Mann-Whitney or Kruskal-Wallis tests. Fisher’s exact test was used to compare proportion among groups. Spearman’s rank test was used for correlations. All tests were two-sided, unadjusted for multiple comparisons, with \( \alpha=0.05 \). GraphPad Prism v9.1 was used for analysis and graphs.
RESULTS

Between April 21st and July 5th, 2021, 58 individuals were included in the HIV<200, 36 individuals in the HIV>500 and 33 as 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 males with a median (IQR) age of 51 years (35; 57). Median (IQR) CD4+ T-cell count and CD4/CD8 ratio at baseline were 173 (117; 257) cells/mm³ and 0.23 (0.15; 0.42) in the HIV<200 group, and 785 (655; 966) cells/mm³ and 1.21 (0.92-1.47) in the HIV>500 group, respectively (p<0.001). Additionally, the percentage of individuals with plasma viral load below 50 copies/ml within the year prior to enrollment was 81% in the HIV<200 group and 100% in the HIV>500 group (P=0.0058).

Vaccine-induced humoral immune responses against SARS-CoV-2

To investigate the magnitude of vaccine-induced antibody responses in PLWH, we assessed anti-S IgG and anti-RBD IgG at enrollment, week 4 and week 8 in all participants to identify potential differences among groups. All groups showed a significant increase of anti-S and anti-RBD IgG titers after first and second dose (Fig. 2, P<0.0001). However, anti-S and anti-RBD IgG in the HIV<200 group were significantly lower than in HIV>500 and control groups, at w4 and w8 (Fig 2A-B, P<0.05). Specifically, the median (IQR) anti-S IgG titer at w8 was 153.6 (26.4; 654.9) in the HIV<200; 245.6 (145; 824) in the HIV>500 and 274.7 (193.7; 680.4) BAU/mL in the control group. Similarly, median (IQR) anti-RBD IgG titer was 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 group (Fig.2).
Next, we classified all individuals into ‘low S-IgG’ and ‘high S-IgG’ according to whether the magnitude of S-specific IgG response at w8 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 ‘low S-IgG’ among PLWH was significantly higher compared to the control group (Fig.2 $P=0.0084$). In terms of clinical variables, ‘Low S-IgG’ individuals were virologically suppressed for a significantly shorter period compared to ‘high S-IgG’ (5 versus 11 years respectively, $P=0.0520$, 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 found any statistically significant differences between groups ($P=0.0640$; supplementary Fig.1), even after comparing individuals with ‘Low S-IgG’ and ‘High S-IgG’ antibodies (Supplementary Fig.1). SARS-CoV-2 vaccination elicited similar anti-S IgA responses among all groups that were successfully boosted with each vaccine dose. However, individuals from the HIV<200 group showed higher IgM responses at w8 than HIV>500 and control groups (Supplementary Fig2).

Overall, these results indicate that PLWH are able to elicit immunogen-specific humoral immune responses after two doses of mRNA vaccination, even though the magnitude of this response is significantly weaker in individuals with <200 CD4+ T-cell counts compared to PLWH with >500 CD4+ T-cell counts, specially if they have been virologically suppressed for a shorter period of time.
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 of a subgroup of individuals at baseline and at week 8, using an assay based on wild-type SARS-CoV-2[5]. At baseline, five participants already had detectable neutralizing activity at baseline (Fig.2), most likely due 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 w8 in all groups (P<0.0001), showing similar neutralization levels (Fig.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 cut-off that might be strong enough to prevent infection[13,18]. We identified 33.3% individuals in the HIV<200 (n=13) compared to 3.7% in the HIV >500 (n=1) and none in the control group showing ID_{50}<1:250 (referred to as ‘low neutralizers’, P=0.0003, Fig.2).

Levels of neutralizing activity positively correlated with anti-S and anti-RBD antibody titers in all groups at w8 (P<0.0001, Spearman’s correlation; rho=0.7715; rho=0.6276, respectively, Fig.3).

Overall, these data suggest that, in the context of PLWH with poor immune reconstitution, one out of three individuals showed low levels of neutralizing antibodies that might be below the protective cut-off against SARS-CoV-2 after two doses of mRNA vaccines.
Vaccine-induced cellular immune responses against SARS-CoV-2

To assess whether PLWH with <200 CD4+ T-cell counts were able to mount SARS-CoV-2-specific T-cell responses despite their impaired humoral responses after vaccination, we measured IFNγ-secreting cells by ELISpot in a subgroup of individuals selected according to their neutralization activity. ‘High neutralizers’ showed a tendency towards an increase in SARS-CoV-2-specific T-cell responses directed against S1 subunit (more than 4-fold increase, \( P=0.0673 \)) from baseline to w8. Notably, we identified statistically 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 (Fig. 4). However, we did not observe statistically significant increases in cellular responses against S2 subunit (Fig. 4; \( P=0.3864 \)), even after exclusion of B82 and B83 peptide pools which cover the fusion peptide and 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, except for the B82 peptide (Fig. 4). Thus, the overall magnitude of cellular responses against S and the RBD regions at w8 were significantly higher in “high neutralizers” when compared to “low neutralizers” (Fig.4, \( P=0.0158 \), 2 versus 0.7-fold increase, respectively).

Regarding SARS-CoV-2 specific T-cell responses against VOCs at w8, we observed a significant loss of IFNγ-secreting cells against Alpha (\( P=0.0010 \)) and Kappa, Delta and B.1.617.3 VOC (\( P=0.0100 \)) in ‘high neutralizers’ (Supplementary Fig.3).

Taken together, these data suggest a lack of vaccine-induced T-cell immunity against SARS-CoV-2 after two doses of mRNA vaccines in the context of PLWH with CD4+ T-cell counts <200 cell/mm³.
DISCUSSION

We conducted a comprehensive analysis of a large cohort of PLWH 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, where approximately one third of individuals induce low IgG levels, reduced in vitro neutralization activity and no vaccine-induced T-cells after four weeks of the second mRNA vaccine dose.

In our cohort, 20% of individuals in the HIV<200 group had detectable HIV-1 viraemia and, importantly, time under ART-suppression was associated with higher anti-S IgG levels after vaccination. This suggests that chronic immune activation/exhaustion associated with incompletely HIV suppression could impact vaccine-induced humoral responses as seen for other vaccinations[26]. However, at the beginning of 2021, SARS-CoV-2 vaccination was prioritized in these individuals rather than delaying vaccination until they were undetectable. Since there is a significant proportion of PLWH worldwide with low CD4+ T-cell counts and persistent viraemia, often suffering from several comorbidities and more severe outcomes for infectious diseases[27], our data might advise clinicians to make an informed risk/benefit decision upon deferring vaccination depending on individual characteristics and incidence status of the pandemic.

Impaired immune responses to pneumococcus[26], influenza[28] and hepatitis A and B[29], are widely described in PLWH. Previous studies have shown that primary responses and the maintenance of long-term serologic memory are affected during HIV-
Infection[30,31]. The inability of CD4+ T-cells to effectively activate B-cells[32] and
the HIV-associated premature exhaustion of B-cells could cause a suboptimal humoral
response to vaccination[33]. Importantly, vaccine responsiveness remain often
diminished despite optimal ART-suppression[26]. Although PLWH with lower CD4+
T-cell counts have received priority access to primary SARS-CoV-2 vaccination, future
studies should address different adjuvants or additional/heterologous vaccinations in
this population.

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[34]. It has been documented that after natural infection,
IgA dominate the early neutralizing humoral response to SARS-CoV-2[35]. In addition,
mRNA SARS-CoV-2 vaccination successfully boosted mucosal IgA response in
convalescent individuals[36]. Here HIV<200 individuals showed comparable titers of
IgA but higher IgM responses than HIV>500 and control groups. If the increase of anti-
S IgM responses observed in HIV<200 group is a consequence of an impaired anti-S
IgG response needs further investigation.

In our study, using a virus isolated during the first COVID-19 wave in Spain -March
2020 harboring the S D614G mutation, 33% of individuals from the HIV<200 group
showed reduced levels of neutralization at w8 after vaccination. These results confirm
the findings recently reported in an Italian cohort of PLWH with different degrees of
immune recovery[37]. 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/or 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[19,38–41]. We showed here an impaired vaccine-induced T-cell immunity against SARS-CoV-2 in PLWH that were both “low S-IgG” and “low neutralizers”. These results are further supported by a recent study showing that the magnitude of SARS-CoV-2-specific T-cell responses after natural infection in PLWH positively correlates with naive CD4+ T-cells and the CD4/CD8 ratio[42]. These findings could be explained in part by the reduced production of IL-2 from antigen-specific CD8+ T-cells in PLWH, which could hinder their proliferative potential and long-term immune memory following natural infection and/or immunization[43]. 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.

Given the impaired humoral and cellular immune responses after two SARS-CoV-2 vaccine doses in a significant proportion of PLWH with less than 200 CD4+ T-cells, our data suggest that these individuals could benefit from close monitoring to prioritize them for alternative strategies aimed at achieving better immunity against SARS-CoV-2 but especially, limiting severe disease outcomes. These strategies could consist on additional doses (homologous or heterologous boosters) together with a close monitoring of antibody levels to detect suboptimal responsiveness, such as what is contemplated in the ongoing European EU funded project RBDCOV (101046118). As
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 recent demonstration of the use of neutralizing monoclonal antibodies, such as the combination of Tixagevimab and Cilgavimab, for preventing SARS-CoV-2 acquisition (Evusheld)[44] suggests that passive antibody prophylaxis could be an approach to consider in PLWH who do not have an adequate immune response to vaccination or cannot be vaccinated against COVID-19 because of severe allergies. Since we identified that PLWH with less than 200 CD4\(^+\) T-cells have an impaired vaccine responsiveness, which is in line with previous studies[37], we propose the use of the recently approved Evusheld in this particular population, which is not currently eligible for this treatment.

This study has some limitations. First, we did not assess immune responses in PLWH with CD4\(^+\) T-cells counts between 200 and 500 cells/mm\(^3\). Second, the latest time-point analyzed in our study was one month after the second vaccine dose. Thus, it is unclear how fast the S-IgG levels wane in these individuals and consequently, long-term data are needed to better guide needs and timings for booster vaccinations in this population. Finally, other functions of antibodies such as ADCC that might also contribute to control of COVID-19 outcomes or activity against more contemporaneous VOC has not been assessed.

In conclusion, our study demonstrated that one out of three PLWH with CD4\(^+\) T-cell counts <200 cells/mm3 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 to prioritize them for additional booster
vaccinations and/or alternative preventive approaches against SARS-CoV-2.

NOTES

Authors contribution
Study conception, design, and funding: BC, RP, NI-U, JC, JGP, JM, BM. Collected
data: CM, SG, SB. Performed humoral experiments, analyzed and interpreted data: EA-
E, NP-L, JM-B, DR-R, DP-Z, MLRC, CA, YA-S, NI-U, SB, BM. Performed T-cell
experiments, analyzed and interpreted data: OB-L, RP, EJ, SC, TE, LR-M, YA-S, GFR-
L, JGP, SB, BM. Manuscript editing: SB, OB-L, EA-E, NP-L, JM-B, DR-R, DP-Z, RP,
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ME, NI-U, JC, JCG, JM, BM. Performed critical revision: BC, RP, JM, BM, SB. All
authors reviewed and approved the final draft of the paper.

FOOTNOTE PAGE:

Conflicts of interest
Unrelated to the submitted work, N.I-U. reports institutional grants from HIPRA,
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Corresponding author information:
Beatriz Mothe
Fight AIDS and Infectious Diseases Foundation
IrsiCaixa AIDS Research Institute
Hospital Universitari Germans Trias i Pujol
Ctra. de Canyet, s/n. 08916 Badalona, Spain
Tel. number: + 34 93 497 88 87
Fax number: + 34 93 465 76 02
e-mail: bmothe@irsicaixa.es
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Table 1: Baseline characteristics of the study cohort at inclusion.

| Characteristic                                      | HIV <200 group | HIV >500 group | Control group | p-value  |
|-----------------------------------------------------|----------------|----------------|---------------|----------|
| Age, years                                          | 52 (40; 56)    | 51 (40; 56)    | 53 (35; 57)   | 0.8631   |
| Male gender, n (%)                                  | 46 (79.3%)     | 29 (80.6%)     | 18 (54.5%)    | 0.0187   |
| HIV route of acquisition, n (%)                     | N/A            | N/A            | N/A           | 0.3771   |
| Sexual contact                                      | 39 (67.2%)     | 25 (69.4%)     | N/A           |         |
| UDVP                                                | 13 (22.4%)     | 10 (27.8%)     | N/A           |         |
| Other                                               | 6 (10.3%)      | 1 (2.8%)       | N/A           |         |
| pVL (log10 copies/ml)                               | 1.30 (1.30; 1.52) | 1.30 (1.30; 1.30) | N/A |         |
| Undetectable pVL, n (%)                             | 47 (81%)       | 36 (100%)      | N/A           | 0.0058   |
| Time on virological suppression, years              | 3.75 (1.09; 14.88) | 10.88 (6.63; 11.42) | N/A | 0.1248   |
| Time from HIV diagnosis to first COVID-19 vaccine, years | 14.08 (1.69; 28.31) | 17.79 (3.50; 28.32) | N/A | 0.1013   |
| Absolute CD4, cells/mm³                              | 173 (117; 257) | 785 (655; 966) | N/A           | <0.0001  |
| CD4/CD8 ratio                                       | 0.23 (0.15; 0.42) | 1.21 (0.92; 1.47) | N/A | <0.0001  |
| CD4 nadir, cells/mm³                                | 64 (22; 110)   | 259 (146; 375) | N/A           | <0.0001  |

N/A: Not applicable
UDVP: intravenous drug users
pVL: plasma viral load
Virological suppression was considered below 50 copies/ml
All values were expressed as median (interquartile range) except when noted
Kruskall-Wallis test was used for comparison between the three groups
Chi-squared test was used for comparison between groups
Mann-Whitney test was used for comparison between groups
Table 2: Baseline characteristics of PLWH with low or high anti-S IgG antibody levels.

Median and IQR from individuals are shown, except for categorical data in which frequency and percentage are presented.

|                                | Low S-IgG n=50 | High S-IgG n=43 | p-value |
|--------------------------------|----------------|-----------------|---------|
| Age, years                     | 51 (40; 57)    | 52 (43; 56)     | 0.7544  |
| Undetectable pVL, n (%)        | 42 (84%)       | 40 (93%)        | 0.2133  |
| Time on virological suppression, years | 5 (1; 22)     | 11 (3; 13)      | 0.0520  |
| Time from HIV diagnosis to first COVID-19 vaccine, years | 14 (3; 29) | 19 (7; 29) | 0.3323 |
| Absolute CD4, cells/mm³        | 254 (132; 663) | 346 (171; 780) | 0.1918  |
| CD4/CD8 ratio                  | 0.83 (0.21; 1.44) | 0.69 (0.25; 1.4) | 0.6267  |
| CD4 nadir, cells/mm³           | 101 (60; 553)  | 115 (23; 257)   | 0.7142  |

All values were expressed as median (interquartile range) except when noted.
Virological suppression was considered below 50 copies/ml.
Mann-Whitney test was used for comparison between groups, except in (₸) where Fisher Exact test was used.

FIGURE LEGENDS

Figure 1. Flow diagram for the prospective observational cohort study according to STROBE statement.

Figure 2. Vaccine-induced humoral immune responses in individuals from our cohort. Antibody binding titers against SARS-CoV-2 S protein (A)- or RBD (B) in individuals in the HIV<200 group, the HIV>500 group and control group at baseline (BSL), at w4 (w4) and at w8 (w8) after vaccination. Median titer of antibodies and IQR shown are quantified in normalized Binding Antibody Units (BAU). Friedman test was used to compare antibody titers at baseline and w8 for each study group and Mann-Whitney test was used to compare median levels of antibody at w8 between groups. C. Proportion of ‘low S-IgG’ individuals according to the magnitude of anti-S IgG response at w8. The percentage of ‘low S-IgG’ (defined as anti-S IgG <193.7 BAU/mL) at w8 is indicated. Fisher’s exact test is used. D. SARS-CoV-2 neutralization titers (ID₅₀
expressed as reciprocal dilution) from individuals belonging to either the HIV<200 group (n=42), the HIV>500 group (n=27) and the control group (n=27) at baseline and at w8. Median titer of neutralization antibodies and IQR are depicted. Individuals with neutralizing capacity at baseline are represented with diamond dots. Grey shaded area shows titers below 250, which are considered as reduced neutralizing activity. Percentage of vaccinated individuals that do not achieve high neutralization capacity (i.e have titers <250) is highlighted in red boxes. Wilcoxon matched-pairs signed rank test was used to compare neutralization titers at BSL and w8 for each study group and Mann-Whitney test was used to compare median levels at w8 between groups.

ns: not significant.

All undetectable levels are represented as 1.

Figure 3. Association between SARS-CoV-2 neutralization titers and levels of binding antibodies at w8.

A. Association between SARS-CoV-2 neutralization titers and levels of binding anti-S IgG antibodies against the virus from individuals analyzed at w8. Spearman’s correlation was used. B. Association between SARS-CoV-2 neutralization titers and levels of binding anti-RBD IgG antibodies against the virus from individuals analyzed at w8. Spearman’s correlation was used.

ns: not significant.

All undetectable levels are represented as 1.

Figure 4. Vaccine-induced T-cell immune responses against the S1 and S2 subunits from the SARS-CoV-2 Spike protein in ‘High and Low neutralizers’ from HIV<200 group. Magnitude of IFN-γ-SFC per million of PBMCs to SARS-CoV-2 peptide pools
covering S1 (A) and S2 (B) subunits in ‘High neutralizers’ individuals at baseline (BSL) and w8 (w8) post-vaccination. Magnitude of IFN-γ-SFC per million of PBMCs to SARS-CoV-2 peptide pools covering S1 (C) and S2 subunits (D) in ‘low neutralizers’ at baseline and at w8 post-vaccination.

ns: not significant.

Figure 5. Comparison of vaccine-induced T-cell responses at w8 between ‘High and Low neutralizers’ from HIV<200 group.

Total magnitude of IFN-γ-SFC per million of PBMCs to S (A) and RBD peptide pools (B) in Low and High neutralizers at w8 post-vaccination. Scatter plots show the median and interquartile ranges. We used the Mann-Whitney test in unpaired samples and the Wilcoxon test in paired samples for statistical analysis.

ns: not significant.
Figure 1

174x88 mm (x DPI)
Figure 2

198x208 mm (x DPI)
Figure 5

(A) Total magnitude IFN-γ SFC/10^6

- S1+S2
  - P=0.01

- 'Low' 'High' neutralizers

(B) Total magnitude IFN-γ SFC/10^6

- RBD
  - P=0.01

- 'Low' 'High' neutralizers