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Similar humoral immune responses against the SARS-CoV-2 spike protein in HIV and non-HIV individuals after COVID-19

Dear Editor,

We have read with interest the article of Venturas et al., who found persons living with HIV (PWH) are not at higher risk of moderate or severe COVID-19 than the general population[1]. The immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in PWH is a matter of controversy and intense research, as HIV infection may impair the immune response to SARS-CoV-2[2]. High levels of neutralizing antibodies against SARS-CoV-2 spike (S) protein are associated with less severe disease and a good prognosis in COVID-19[3]. These antibodies against the SARS-CoV-2 S protein block the virus union to its cellular receptor, the angiotensin-converting enzyme 2 (ACE2) receptor[2].

Thus, it is critical to determine whether the anti-SARS-CoV-2 neutralizing antibody response is impaired in PWH[2]. This study aimed to characterize plasma antibodies against SARS-CoV-2 S protein in PWH and CTRLs recovered from COVID-19.

We performed a cross-sectional study in 91 PWH from the Cohort of the Spanish HIV Research Network (CoRIS) seropositive for SARS-CoV-2 and with plasma specimens collected from April 1, 2020, to September 30, 2020[4]. We also included HIV-uninfected CTRLs seropositive for SARS-CoV-2 with plasma specimens stored in the National center for Microbiology Instituto de Salud Carlos III. Both groups were matched for age and time since initiation of symptoms and were not vaccinated against SARS-CoV-2. The Ethics Committee of Hospital General Universitario Gregorio Marañón approved the study (Ref# 162/20).

Blood samples were collected by venipuncture in EDTA tubes and were sent the same day to the Spanish HIV BioBank, where plasma samples were obtained and stored at −80 °C. These samples were sent to the Instituto de Salud Carlos III for its analysis. We used immunoassays to evaluate the antibody titer against the SARS-CoV-2 S protein, which gives us the area under the curve (AUC) of IgG, IgM, and IgA titration curves. Besides, we assayed the capacity of the antibodies to inhibit the binding of the soluble ACE2 receptor to S protein (see Supplemental file 1).

The differences between groups were calculated by the Mann-Whitney U test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. Generalized Linear Models (GLM) with a gamma distribution (log-link) adjusted by age, gender, and COVID-19 disease severity were used to evaluate the differences in plasma anti-SARS-CoV-2 S protein antibody levels (IgG, IgM, and IgA) between groups. The inhibition of ACE2 binding to the S protein (inhibition percentage, y-axis) and the titers of plasma anti-SARS-CoV-2 S protein antibodies (sum of AUCs of IgG, IgM, and IgA titration curves, x-axis) were plotted according to a semilog line, and Pearson's correlation coefficient (r) was calculated. Then, GLM tests were used to assess if regression slopes in PWH and CTRLs were differents by analyzing the interaction between the groups (PWH vs. CTRLs) with the sum of AUCs and inhibition percentages. Statistical analysis was performed with GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA, USA) and IBM SPSS Statistics 25.0 (SPSS INC, Armonk, NY, USA). The level of significance was two-tailed and defined as $p < 0.05$ (two-tailed).

The study population included 91 PWH – fully described elsewhere[4] – and 21 CTRLs, whose characteristics are shown in Table 1. Concerning COVID-19, 92.3% PWH had asymptomatic or mild COVID-19 disease, 7.7% were hospitalized, and the median time from symptoms to plasma collection was 11 weeks. CTRLs had similar characteristics to PWH, except for gender.

No significant differences were found between groups in plasma levels of different classes of immunoglobulins against SARS-CoV-2 S protein [IgG ($p = 0.414$; Fig. 1A), IgM ($p = 0.862$; Fig. 1B), and IgA ($p = 0.134$; Fig. 1C)], and percentages of inhibition of ACE2 binding to the S protein ($p = 0.237$; Fig. 1D). Adjusted regression analysis also found no significant differences (Supplemental Table 1). Furthermore, we found solid and similar correlations between total plasma antibody titers against SARS-CoV-2 S protein and the percentage of inhibition of ACE2 binding to the S protein

Table 1
Epidemiological and clinical characteristics of SARS-CoV-2 infected patients.

Variable	Control group	HIV group	*p*-value
No.	21	91	
Demographic data			
Male sex at birth – No./with data (%)	13 (61.9%)	85 (93.4%)	< 0.001
Age - Median (Q1; Q3) – yr.	42.3 (38.9; 48.8)	44.2 (36.8; 51.6)	0.902
COVID-19 data			
Severity status (asymptomatic or mild) – No./with data (%)	18 (85.7%)	84 (92.3%)	0.277
Hospital admission – No./with data (%)	3 (14.3%)	7 (7.7%)	0.340
Time from symptoms - Median (Q1; Q3) – wk.	12.3 (11.1; 19.7)	11 (8.1; 15.4)	0.106
Oxygen-therapy – No./with data (%)	3 (14.3%)	6 (6.6%)	0.340
HIV infection data			
Mechanism of HIV acquisition – No./with data (%)			
Men having sex with men	-	68 (74.7%)	-
Heterosexual	-	20 (22%)	-
Injection drug use	-	1 (1.1%)	-
Other	-	2 (2.2%)	-
Age of HIV diagnosis - Median (Q1; Q3) – yr.	-	36.4 (28.1; 43.6)	-
Time with HIV infection - Median (Q1; Q3) – yr.	-	6.2 (3.3; 11.5)	-
Prior AIDS-defining conditions – No./with data (%)	-	11 (12.1%)	-
Age - Median (Q1; Q3) – yr.	-	45 (36.9; 46.9)	-
Last CD4+ count			
Median (Q1; Q3) - cells/mm3	-	696.5 (491.5; 939)	-
Distribution – No./with data (%)			
< 350	-	9/84 (10.7%)	-
350–499	-	13/84 (15.5%)	-
≥ 500	-	62/84 (73.8%)	-
Last HIV-RNA load ≤ 50 copies/mm3 – No./with data (%)	-	80 (94.1%)	-
Antiretroviral therapy – No./with data (%)	-	88 (96.7%)	-
Antiretroviral therapy (N[t]RTI backbone) – No./with data (%)			
TAF/FTC	-	40 (44%)	-
ABC/3TC	-	25 (27.5%)	-
TDF/FTC	-	5 (5.5%)	-
Antiretroviral therapy (third drug)			
NNRTI	-	48 (52.7%)	-
Protease inhibitor	-	4 (4.4%)	-
Integrase inhibitor	-	51 (56%)	-

Abbreviations: PWH. People with HIV; Q1. 1st quartile; Q3. 3rd quartile; N(t)RTI. nucleoside/nucleotide reverse transcriptase inhibitors; TAF. tenofovir alafenamide; FTC. emtricitabine; ABC. abacavir; 3TC. lamivudine; TDF; tenofovir disoproxil fumarate; NNRTI. non-nucleoside reverse transcriptase inhibitors.

in CTRLs ($r = 0.580$; $p = 0.005$; Fig. 1E) and PWH ($r = 0.548$; $p < 0.001$; Fig. 1F). No differences were found between the regression slopes of the two study groups ($p = 0.849$).

Several studies have reported that PWH usually shows poor antibody response to other viruses or viral vaccines[5–7], raising concerns about whether they can mount an adequate humoral response against SARS-CoV-2. This issue is relevant since high antibody titers against the SARS-CoV-2 S protein correlate with virus neutralization and protection[3]. Our study shows that PWH and CTRLs who recovered from COVID-19 display a similar antibody response against the S protein. To detect neutralizing antibodies, we used a stabilized trimeric S protein in its native pre-fusion conformation. The suitability of our assay was confirmed by the strong correlation between the antibody titers and their capacity to inhibit the interaction S protein-ACE2 receptor.

Our data agree with recently published results showing comparable anti-SARS-CoV-2 neutralizing antibody levels between PWH under effective antiretroviral therapy (ART) and HIV-uninfected individuals[8,9]. Succesful HIV suppression seems to be crucial for developing an adequate humoral immune response. In our study, almost all HIV patients analyzed were on ART, with good clinical, virological, and immunological control, which may have contributed to similar anti-SARS-CoV-2 antibody titers between PWH and CTRLs. We analyzed the antibody titers against the SARS-CoV-2 S protein and percentages of inhibition of ACE2 binding to the S protein according to $CD4^+$ strata (< 350, 350–500, > 500 cells/mm^3), and we did not find significant differences (data not shown). In contrast, lower neutralizing antibody titers against SARS-CoV-2 were found in PWH than in HIV-uninfected individuals recovering from COVID-19 by Spinelli et al.[10], although its sample size was three times lower than in our study. Differences in the characteristics of the study cohorts (sample size, ethnicity, age, sex, COVID-19 severity, percentage of people with unsuppressed viral loads, among others), study design, or assays for antibody characterization may explain these conflicting results.

In conclusion, no differences in quantitative and qualitative SARS-CoV-2-specific immune humoral response were found between well-controlled PWH and CRTLs after recovery from COVID-19. This finding suggests that PWH are not an at-risk population for this infection and are potentially good vaccination responders.

Contribution

- Study conception and design: Salvador Resino, Juan Berenguer, and Isidoro Martínez.
- Acquisition of data: all authors.
- Laboratory procedures: María Martín-Vicente, and María José Muñoz-Gómez.
- Analyses and interpretation of data: Salvador Resino and Isidoro Martínez.
- Drafting the article: Salvador Resino and Isidoro Martínez.
- Critical revision of the article: Juan Berenguer.
- Funding acquisition: Salvador Resino and Juan Berenguer.
- All authors have read and approved the final manuscript.

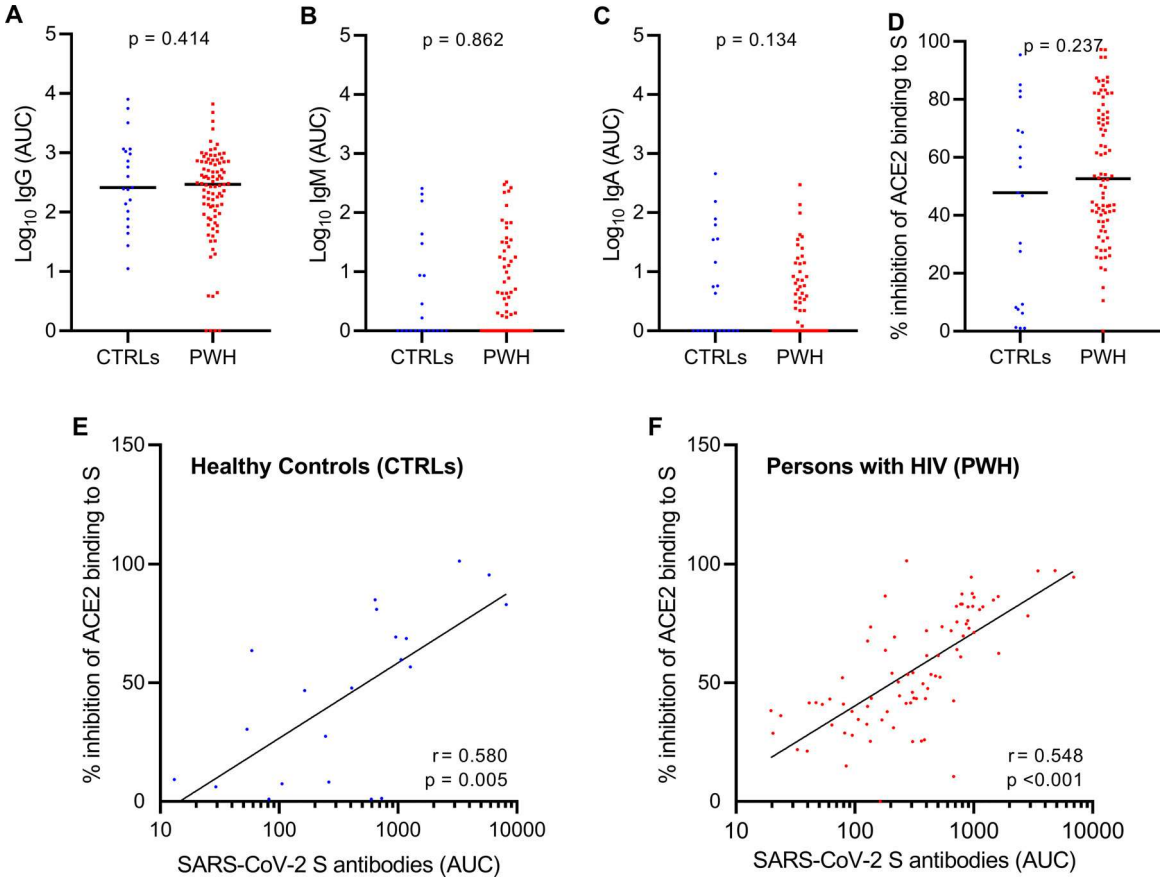

Fig. 1. Plasma levels of antibody against SARS-CoV-2 S protein (A–C) and percentages of inhibition of ACE2 receptor binding to the S protein (D). Correlation between antibody levels against SARS-CoV-2 S protein (sum of the AUC of IgG, IgM, and IgA) and percentages of inhibition of ACE2 receptor binding to the S protein (E and F).
Statistics: Differences were calculated by the Mann-Whitney U test, and medians were represented by a horizontal bar. Correlation analysis was performed using the Pearson test.
Abbreviations: AUC, the area under the curve; ACE2, angiotensin-converting enzyme 2; CTRLs, HIV-uninfected patients, PWH, persons living with human immunodeficiency virus; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus; IgG, anti-SARS-CoV-2 S IgG; IgM, anti-SARS-CoV-2 S IgM; IgA, anti-SARS-CoV-2 S IgA.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All participants gave their consent before enrollment. The Ethics Committee of Hospital General Universitario Gregorio Marañón approved the study (Ref# 162/20).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.002.

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Divergent humoral responses in mild to moderate SARS-CoV-2 infection over time – indication of persistence of the virus?

Dear Editor,

Serum antibodies are an important pillar of the immune response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. With earlier data, we have shown that SARS-CoV-2 IgG and IgA antibody responses are both, gender dependent and characterized by a declining antibody concentration early on[1]. We can now show that antibodies, especially against spike protein (S), remain detectable for more than one year in most persons after PCR confirmed mild to moderate COVID-19, despite the fact that a relevant decline can be observed. We here present the extended longitudinal profile of IgG and IgA against S and of IgG against nucleocapsid protein (N) for more than one year (the cohort was initiated during the first infection wave in Switzerland in March 2020).

The study includes outpatients with a history of positive SARS-CoV-2 PCR, *i.e.* a mild to moderate disease course. The total cohort comprises 278 individuals (12.0–91.2 years, median = 51.2, IQR = 25.8; 59.5% females), of which 53 (24.8–91.2 years, median = 55.8, IQR = 13.9; 41% females) were followed for 14 months (supplementary Table 1). The study is registered in the Swiss COVID-19 database (https://swissethics.ch/covid-19/approved-projects; K2) and was approved by the regional ethics committee (ID2020–00,941). PCR analysis of stool and nasopharyngeal swabs were performed together with blood draws every week in a first month and then after another four weeks in the second month; this course was repeated if patients consented. All SARS-CoV-2 ELISA (anti-S IgG and IgA, Euroimmun, Lübeck, Germany; anti-N IgG, Epitope Diagnostics, San Diego, USA) were run on an automated DSX ELISA processor (Dynex Technologies) according to the recommendations of the manufacturers. We defined an OD ratio of 11 (anti-S IgG) or 9 (anti-S IgA) as the upper threshold of the dynamic range, since the assays saturate above these points[2]. Statistical definitions, analysis and visualizations were based on or performed with software R using the implemented statistical tests and the packages “tidyverse” and “ggplot2”[3].

During the initial 4 months after a positive PCR result, 94.2% of participants showed quantifiable evidence of seroconversion, while 5.8% did not (Fig. 1A–C). Upon their first visit (median 6 weeks after positive PCR; 95% CI 0.43 weeks) 11.9% (33 / 278), 21.6% (60 / 278) and 24.5% (68 / 278) had not developed measurable anti-S IgG, anti-S IgA or anti-N IgG, respectively. Furthermore, 66.9% of participants displayed quantifiable antibody concentrations for all three entities evaluated. Remarkably, all long-term sub cohort participants presented at least one quantifiable antibody entity at all time points until their last visit, while only 49% showed quantifiable antibody concentrations in all three entities. Note that study participants with no initially detectable antibodies against SARS-CoV-2 (5.8%) did not participate in the long-term sub cohort.

The statistically significant gender-associated difference in the antibody concentrations observed earlier persist for the first 3 months; thereafter, gender-associated differences are no longer observed[1]. In addition, a significant ($p < 2.1e{-}08$) age dependent difference in antibody concentrations becomes apparent at weeks 22 to 26 (Fig. 1D). Individuals younger than 54 years of age tend to show lower antibody concentrations than their older counterparts; this was also observed in other studies[4].

While antibody concentrations may have complex kinetics[5], we categorized the anti-S antibody longitudinal courses based on the slope of the robust regression line (Fig. 2). We identified two statistically distinctive patterns of antibody dynamics for anti-S IgG and IgA: declining antibody concentrations (decrease of anti-S IgG levels, average slope: −0.045 (±0.037) OD ratio/week, $n = 47$; decrease of anti-S IgA levels, average slope: −0.032 (±0.052) OD ratio/week, $n = 19$) and increasing antibody concentrations (increase of anti-S IgG levels, average slope: +0.029 (±0.020) OD ratio/week, $n = 6$; increase of anti-S IgA levels, average slope: +0.053 (±0.066) OD ratio/week, $n = 34$).

The majority (89%) of the long-term sub cohort showed declining anti-S IgG antibody concentrations, while a small subgroup (11%) showed increasing antibody concentrations over time (Fig. 2B). An even higher proportion of increasing antibody concentrations was observed with the individual courses of anti-S IgA antibodies (36% declining and 64% increasing antibody concentrations). As there are substantially more individual anti-S IgA increases than decreases, this might indicate an underlying mechanism of IgA stimulation. The detection of SARS-CoV-2 material in some stool samples early during the observation period might be hinting at such a stimulatory exposure (supplementary Table 2). Considering all results of our observation, one might therefore conclude that IgA antibodies might provide a more persistent and more stable defense against SARS-CoV-2 than IgG[6,7].

According to the current understanding, one would have to expect a continuous decrease in antibody concentration - in the absence of the antigen - after an initial increase[5]. Our current data, however, describe a secondary increase in anti-spike IgG in a few and in IgA in many more patients. If this increase was due to re-infection, a much steeper increase (*i.e.* a booster response) could be expected to be observed, at least temporarily[8]. In addition, nasopharyngeal swabs and stool samples for PCR testing were taken at every visit, but none of them were found to be positive in any of the individuals within the long-term sub-cohort; obviously, this observation does not allow to rule out a potential re-infection or re-exposure during the observation period with certainty. However, it seems at least to rule out persistence of a high viral load in the nasopharynx and the gut within this group. But even non-detectable persistence of virus particles might have provided sufficient antigen to induce the observed response, preventing waning of antibodies. This would be compatible with findings of coronavirus particles in the small bowel of covalescent study