SARS-CoV-2 adaptive immunity in nursing home residents following a third dose of the Comirnaty® COVID-19 vaccine

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ABSTRACT

A third Comirnaty® vaccine dose increased SARS-CoV-2-receptor binding domain antibody levels (median of 93-fold) and neutralizing antibody titers against Wuhan-Hu-1 (median, 57-fold), Beta (median, 22-fold), Delta, (median, 43-fold) and Omicron (median, 8-fold) variants, particularly in SARS-CoV-2-naïve individuals, but had a negligible impact on S-reactive T-cell immunity in nursing home residents.

Keywords: SARS-CoV-2, Comirnaty® COVID-19 vaccine third dose, SARS-CoV-2-S antibodies, SARS-CoV-2-S T cells, neutralizing antibodies, nursing home residents.

INTRODUCTION

Health organizations and drug regulatory agencies in Western countries recently endorsed the use of an additional dose of mRNA COVID-19 vaccines for highly vulnerable population groups, including the elderly [1]. This recommendation is strongly supported by the reduction in vaccine effectiveness a few months following a two-dose schedule against circulating SARS-CoV-2 variants of concern that has been documented in the general population [2,3]. Vaccine-elicited adaptive immunity appears to wane more substantially and at a faster rate in elderly individuals with frailty and comorbidities compared to younger healthy controls [4]. In this context, there is limited information as to the effect of a third mRNA COVID-19 vaccine dose on SARS-CoV-2 adaptive immune responses in this population group [5]. Here, as part of the "Monitoring of antibody responses following SARS-CoV-2 vaccination in nursing homes of the Valencian Community" program launched by the Valencian Community (VC) government (COVID-19 vaccine research program -ProVaVac-) [6] we assessed SARS-CoV-2-Spike (S) targeting antibody and T-cell responses in nursing home residents after receipt of a third dose of the Comirnaty® COVID-19 vaccine.

METHODS

The current observational cohort study was carried out under the epidemiological surveillance competences of the Valencia Government Health Department (Law 16/2003/May 28 on Cohesion and Quality of the National Health System, and Law 10/2014/ December 29 on Public Health of the Valencian Community), without requiring informed consent, as approved by the institutional ethical review board of the Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana FISABIO, (Valencia, Spain). A total of 143 residents (107 female; median age, 80 years; range, 63-99 and with a median Charlson comorbidity index of 7 (range 1-14) residing in 4 randomly selected nursing home residences from the Valencian Community were recruited between October and November 2021. Whole blood was obtained at baseline, following completion of the two-dose Comirnaty® vaccine schedule (median, 209 days; range, 226–247), and at a median of 26

days (range, 20-43) after receiving the third vaccine dose (3D). SARS-CoV-2-receptor binding domain (RBD) and nucleocapsid (N)-reactive total antibodies were measured by the Roche Elecsys® Anti-SARS-CoV-2 S and Elecsys® Anti-SARS-CoV-2 N assays (Roche Diagnostics, Pleasanton, CA, USA), respectively. Values ≥0.4 BAU/ml and ≥1.0 Cut-off index (COI) were considered as positive results, respectively.

Neutralizing antibodies (NtAb) targeting the S protein were measured using a GFP-expressing vesicular stomatitis virus pseudotyped with the Wuhan-Hu-1 G614, Beta, Delta, and Omicron variants, as previously described [7]. SARS-CoV-2-S specific-IFNγ-producing $CD4^+$ and $CD8^+$ T-cell immunity were enumerated by whole-blood flow cytometry for intracellular cytokine staining (ICS) (BD Fastimmune, Becton Dickinson and Company Biosciences, San Jose, CA), as previously reported [6,8]. Further details are given in the Supplementary Material, and the antibodies used are listed in Supplementary Table 2.

Frequency comparisons for categorical variables were carried out using the Fisher exact test. Differences between medians were compared using the Mann–Whitney U-test or the Wilcoxon test, as appropriate. Two-sided exact *P*-values were reported. A *P*-value <0.05 was considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

RESULTS

At baseline, 76 and 67 participants were SARS-CoV-2-naïve and experienced, respectively, according to historical records and absence or presence of anti-N total antibodies. No participants developed SARS-CoV-2 vaccine breakthrough infection between sampling times. A total of 142 out of 143 participants had detectable SARS-CoV-2-RBD total antibodies at both sampling times. As shown in Figure 1A, median antibody levels at baseline were significantly higher in SARS-CoV-2-experienced (median, 3,736 BAU/ml; range, 19–255,873) than in SARS-CoV-2-naïve individuals (median, 110 BAU/ml; range, 3–5,985). Antibody levels increased in 113 residents (80%), decreased in 14 (9.7%),

and remained unchanged in 16 (11%) following receipt of a third vaccine dose; Charlson comorbidity index was similar (median 7) across the 3 comparison groups (*P*=1.00). As depicted in Figure 1B, overall, antibody levels increased significantly (a median of 93-fold) between sampling times, more markedly (*P*<0.0001) in SARS-CoV-2-naïve (median, 161-fold) than in experienced residents (median, 14-fold). NtAb activity against SARS-CoV-2-S pseudotyped Wuhan-Hu-1, Beta and Delta virus variants was examined in 47 randomly selected residents (36 were SARS-CoV-2-naïve and 11 experienced). Of these, 24 paired samples from 15 naïve and 9 experienced participants could be assessed for NtAb activity against the Omicron variant. As shown in Supplementary Table 1, NtAb against Wu-Hu-1 G614 were measurable in 31/47 residents at baseline (22 naïve and 9 experienced) and in 43/45 after 3D (32 naïve and 11 experienced). For the Beta variant, 15/47 had detectable NtAb titers at baseline (7 naïve/8 experienced) versus 41/45 after 3D (30 naïve and 11 experienced). For the Delta variant NtAb were observed in 24/47 at baseline (16 naïve and 8 experienced) and 43/45 after 3D (32 naïve and 11 experienced) and for the Omicron variant, NtAb were detected in 3/24 at baseline (all experienced) and 19/24 after 3D (11 naïve and 8 experienced).

As shown in Figure 1C, overall, IC_{50} NtAb titers against all variants increased significantly (*P*<0.0001) after 3D, most notably against Wu-Hu-1 G614 (median, 57-fold) followed by the Delta (median, 43-fold), Beta (median, 22-fold) and Omicron (8-fold) variants. Median NtAb titer increase was higher in naïve than in experienced residents (82-fold vs. 4-fold for Wuhan-Hu-1 G614; *P*=0.03; 23-fold vs. 5-fold for Beta; *P*=0.17; 50-fold vs. 2-fold for Delta; *P*=0.04, and 13-fold vs. 2.6-fold for Omicron; *P*=0.25) (Supplementary Figure 1).

T-cell immunity data were obtained for 26 randomly selected residents: 17 SARS-CoV-2-naïve and 9 experienced. SARS-CoV-2-S specific, IFNγ-producing $CD4^+$ T cells were detected in 23 and 22 residents at baseline and after 3D, respectively. In turn, SARS-CoV-2-S-specific, IFNγ-producing $CD8^+$ T cells were detectable in 23 and 21 residents, respectively. After 3D, a slight but not statistically significant increase in $CD4^+$ T-cell frequencies was observed in SARS-CoV-2-naïve residents, while

$CD8^+$ T-cell frequencies decreased significantly (Figure 1E). In contrast, no significant differences in SARS-CoV-2-S specific, IFNγ-producing $CD4^+$ or CD8+ T cell levels were observed SARS-CoV-2-experienced individuals (Figure 1F).

DISCUSSION

Several important findings arose from our study. First, in notable contrast to naïve residents, SARS-CoV-2-experienced residents maintained high plasma levels of anti-RBD total antibodies (median, 3,736 BAU) approximately 7 months after completion of the regular two-dose Comirnaty® COVID-19 vaccine schedule. This could potentially translate into a high level of protection against vaccine breakthrough infections, according to recent estimates [9,10]. Moreover, baseline plasma from most SARS-CoV-2-experienced residents displayed neutralizing activity against the Wu-Hu-1 G614 variant and to a lesser extent against Beta, Delta and Omicron variants, unlike in SARS-CoV-2-naïve residents. Second, anti-RBD antibody levels and NtAb titers increased notably against all variants (more modestly against Omicron) after receipt of the third vaccine in most residents, as previously shown in over 60-year-olds [5-11] yet the magnitude of such an increase was clearly more modest (5–24-fold lower) in SARS-CoV-2 experienced residents compared to virus naïve ones. Third, most residents had detectable T-cell responses at baseline. Nevertheless, the impact of a third vaccine dose on frequencies of SARS-CoV-2-S specific T cells was negligible, in both experienced and naïve residents. This observation needs to be verified by using different platforms for T-cell immunity assessment. In addition, this finding must be interpreted with caution due to the low number of participants examined, absence of follow-up samples (peak levels could have been reached at later times after 3D sampling) and the lack of standardization of the flow cytometry assay employed.

Despite its relatively limited sample size, and lack of a control group, our study convincingly proves that receipt of a third Comirnaty® vaccine dose robustly boosts SARS-CoV-2-S specific antibody responses in elderly nursing home residents, particularly SARS-CoV-2-naïve ones. How this effect translates into protection against SARS-CoV-2 infection and COVID-19 in this population group needs

to be assessed [12]. Our data regarding administering additional doses of mRNA vaccines to elderly nursing home residents in the near future suggest that SARS-CoV-2-experienced individuals may display lower-magnitude booster effects than virus-naïve individuals.

NOTES

AUTHOR CONTRIBUTIONS

EG, EA, IT, and MJA carried out anti-RBD antibody and T-cell immunity assays and analyzed the data; JZ, LR, ARM carried out neutralization assays and analyzed the data. JSB, SP, JS-P, JD-D, IC, and FG-C, methodology and data validation. DS, HV, RL, are responsible for the vaccine rollout program at the Valencian Community. RG and DN, conceptualization, data analysis, and writing the original draft. All authors reviewed the final version of the manuscript.

ACKNOWLEDGMENTS

We are grateful to the Vice-presidency and Ministry of Equality and Inclusive Policies of the Valencia Community, the Corporate Association of Residences and Services for People with Dependency of the Valencian Community (AERTE), the Valencia Health System nursing home departmental committees, and the staff and residents of the participant nursing homes for their collaboration in developing the ProVaVac program. We are also grateful to Ana Berenguer, General Director of Analysis and Public Policies of the Presidency of the Generalitat. Eliseo Albert (Juan Rodés Contract; JR20/00011) Estela Giménez (Juan Rodés Contract, JR18/00053) and Ignacio Torres (Río Hortega Contract; CM20/00090) hold contracts funded by the Health Institute Carlos III (co-financed by the European Regional Development Fund, ERDF/FEDER). Ron Geller holds a Ramon y Cajal fellowship from the Spanish Ministerio de Economía y Competitividad (RYC-2015-17517).

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FINANCIAL SUPPORT

This research work was supported by the European Commission NextGenerationEU fund, through CSIC's Global Health Platform (PTI Salud Global) to RG and IC.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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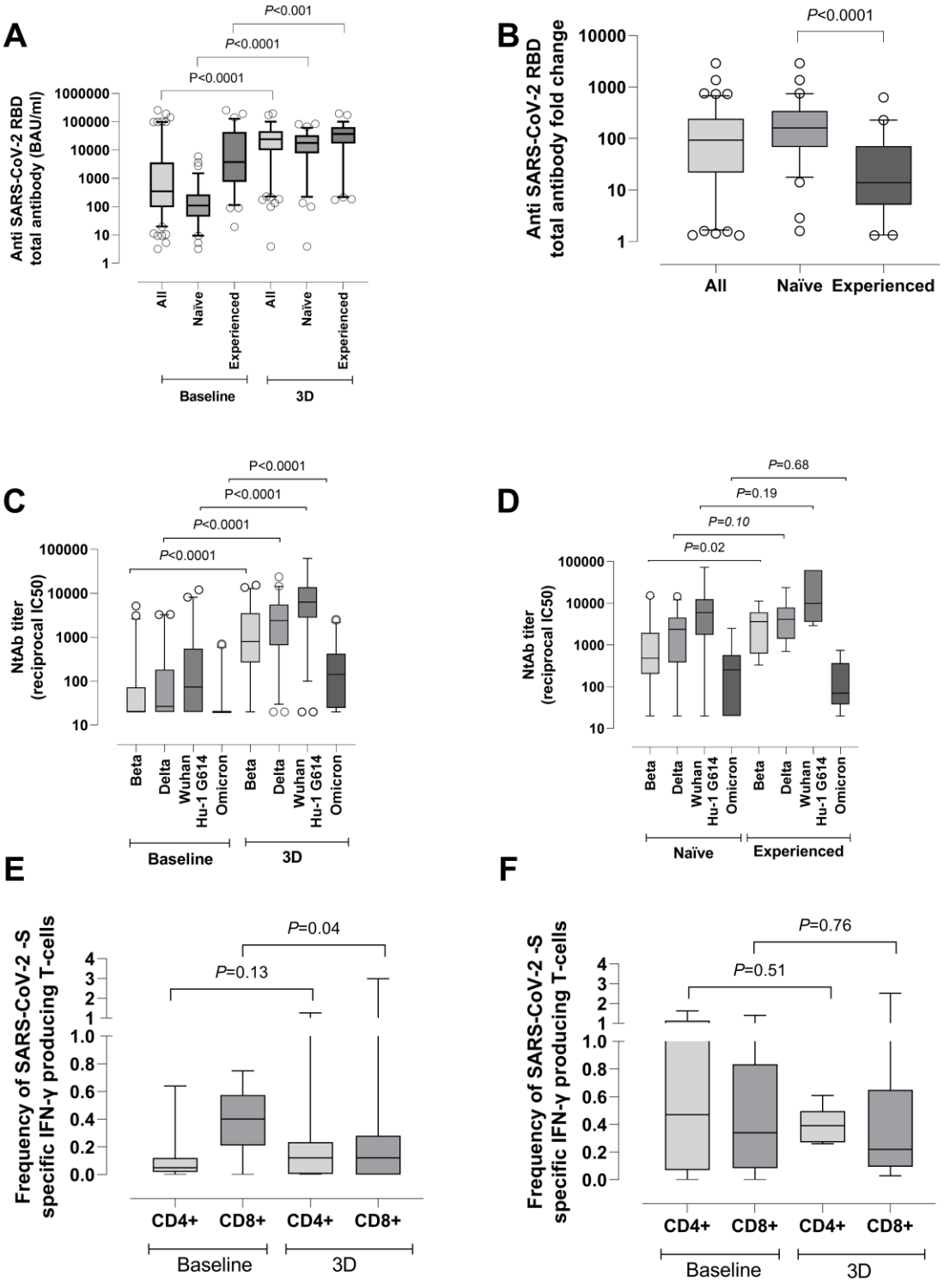
FIGURE LEGENDS

Figure 1. SARS-CoV-2-Spike (S)-reactive antibody and T-cell responses at baseline (median of 7 months after full vaccination with two doses) and at a median of 26 days after receipt of the third Comirnaty® vaccine dose (3D) in nursing home residents. (A) Box Whisker plots depicting SARS-CoV-2-receptor-binding domain (RBD) total antibody levels (in binding antibody units [BAU] per mL). (B) Box Whisker plots showing the fold-change variation in antibody levels quantified after 3D vs. at baseline in SARS-CoV-2-naïve and experienced participants. (C) Box Whisker plots depicting Neutralizing antibody (NtAb) titers against pseudotype viruses carrying the Wuhan, Beta, Delta and Omicron SARS-CoV-2 S protein. (D) Box Whisker plots showing the NtAb titer after 3D according to participants' SARS-CoV-2 infection status. Box Whisker plots depicting SARS-CoV-2-S-specific, IFNγ-producing $CD4^+$ and $CD8^+$ T-cell frequencies at baseline and after 3D in SARS-CoV-2-naïve (E) and experienced individuals (F). *P* values for statistical comparisons are shown.

Figure 1


A
P<0.001
P<0.0001
P<0.0001
Anti SARS-CoV-2 RBD total antibody (BAU/ml)
1000000
100000
10000
1000
100
10
1
All
Naïve
Experienced
All
Naïve
Experienced
Baseline
3D
B
P<0.0001
Anti SARS-CoV-2 RBD total antibody fold change
10000
1000
100
10
1
All
Naïve
Experienced
C
P<0.0001
P<0.0001
P<0.0001
P<0.0001
NtAb titer (reciprocal IC50)
100000
10000
1000
100
10
Beta
Delta
Wuhan Hu-1 G614
Omicron
Beta
Delta
Wuhan Hu-1 G614
Omicron
Baseline
3D
D
P=0.68
P=0.19
P=0.10
P=0.02
NtAb titer (reciprocal IC50)
100000
10000
1000
100
10
Beta
Delta
Wuhan Hu-1 G614
Omicron
Beta
Delta
Wuhan Hu-1 G614
Omicron
Naïve
Experienced
E
P=0.04
P=0.13
Frequency of SARS-CoV-2 -S specific IFN-γ producing T-cells
4
3
2
1
1.0
0.8
0.6
0.4
0.2
0.0
CD4+
CD8+
CD4+
CD8+
Baseline
3D
F
P=0.76
P=0.51
Frequency of SARS-CoV-2 -S specific IFN-γ producing T-cells
4
3
2
1
1.0
0.8
0.6
0.4
0.2
0.0
CD4+
CD8+
CD4+
CD8+
Baseline
3D