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BRIEF REPORT

Neutralizing Response Against SARS-CoV-2 Variants 8 Months After BNT162b2 Vaccination in Naive and COVID-19–Convalescent Individuals

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We have investigated the evolution of the neutralizing response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants at 8 months after Pfizer-BNT162b2 vaccination in coronavirus disease 2019 (COVID-19)–naive (n = 21) and COVID-19–convalescent (n = 21) individuals. Neutralizing levels declined for all variants (range 2- to 3.7-fold). Eight months after vaccination, a significant proportion (4/21) of naive individuals lacked detectable neutralizing activity against the highly transmissible SARS-CoV-2 delta variant. In the convalescent group, the impressive high initial humoral response resulted in detectable neutralizing antibody levels against all variants throughout this period.

Keywords. SARS-CoV-2; COVID-19; vaccine; neutralizing antibodies; variant of concern.

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly protective against severe forms of coronavirus disease 2019 (COVID-19) and its deployment has enormously helped to control the spread of the pandemic [1, 2]; however, the selection of SARS-CoV-2 variants associated with an increased transmissibility can also determine immune escape to neutralizing antibodies induced by natural infection or vaccination, thus jeopardizing pandemic control. Furthermore, waning of vaccine efficacy has been reported, and breakthrough infections in vaccinated individuals have been correlated to low levels of neutralizing antibodies [3]. In this study, we have aimed to investigate the evolution of the neutralizing antibody response against SARS-CoV-2 variants of concern (VoC) at 8 months after vaccination

METHODS

Participants

In this study we included COVID-19–naive (n = 21) and COVID-19–convalescent (n = 21) health care workers from the Hospital Universitario 12 de Octubre in Madrid, Spain. The 2 groups were part of a follow-up study (Solidarity II cohort, institutional review board approval reference CEIm 20/157) and were recruited after informed consent and randomly selected among those with serum samples available for the study period. Mean age was 49 and 48 years for the convalescent and naive groups, respectively. All infections in convalescent individuals took place during the epidemic wave of COVID-19 affecting Madrid during March–April 2020, and all had a mild clinical evolution. All participants were vaccinated in January–February 2021 with 2 doses of the Pfizer-BNT162b2 vaccine 21 days apart [4]. Blood samples were obtained at 61 days (range 42–77 days) and 242 days (range 238–252 days) after the first dose in the convalescent group and at 67 days (range 49–97 days) and 241 days (range 228–252 days) in the naive group.

ELISA Anti-RBD Immunoglobulin G

Anti–receptor-binding domain (RBD) immunoglobulin G (AbRBD) titers were determined by an electrochemiluminescence commercial assay (Elecsys Anti-SARS-CoV-2; Roche Diagnostics) and were converted to World Health Organization international standard binding antibody units and expressed as BAU/mL following the manufacturer instructions.

Production of SARS-CoV-2 Pseudotyped VSV and Neutralization Assays

Neutralization activity was tested by using a SARS-CoV-2-pseudotyped recombinant vesicular stomatitis virus-luciferase (PSV) system. PSV was produced following previously published protocols [5, 6]. The expression vector encoding SARS-CoV-2 spike protein corresponding to the Wuhan-Hu-1 sequence was kindly provided by J. Garcia-Arriaza (Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Madrid, Spain). The SARS-CoV-2 spike mutant D614G was generated by site-directed mutagenesis. SARS-CoV-2 variant B.1.1.7 (GISAID: EPI_ISL_608430), SARS-CoV-2 variant P.1 (GISAID: EPI_ISL_833140), SARS-CoV-2 variant B.1.351 (GISAID: EPI_ISL_712096), and SARS-CoV-2 variant B.1.617.2 (GISAID: EPI_ISL_1970335) were synthesized and cloned into pcDNA3.1 by GeneArt technology (Thermo

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Fisher Scientific). Serum samples were heat-inactivated at 56°C for 30 minutes and tested at dilutions 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, and 1:5120. Pseudotyped viruses were normalized for infectivity to a multiplicity of infection of 0.5–1 and incubated with the dilutions of serum samples at 37°C for 1 hour in 96-well plates. After the incubation time, 2×10^4 Vero E6 cells were seeded onto the virus-plasma mixture and incubated at 37°C for 24 hours. Cells were then lysed and assayed for luciferase expression. Neutralizing titer 50% (NT_{50}) was calculated using a nonlinear regression model fit with settings for log inhibitor versus normalized response curves, in GraphPad Prism version 8, and is expressed as the reciprocal dilution. Means of AbRBD and NT_{50} titers were calculated as geometric mean titers (GMT). Statistical significance among titers was calculated using Wilcoxon matched-paired signed rank test or multiple comparisons 1-way (ANOVA) Friedman test with Dunn correction in GraphPad Prism version 8.

RESULTS

Results are summarized in Figure 1. Vaccination in COVID-19–convalescent individuals induced a much higher level of both binding and neutralizing antibodies as compared with COVID-19–naive individuals at 2 months postvaccination (16.7- and 6.3-fold in AbRBD and NT_{50} against the ancestral sequence, respectively; both $P < .0001$). The beta VoC exhibited the highest neutralizing reduction: 2.5-fold in convalescents and 4.5-fold in naive individuals.

Eight months after the first dose, AbRBD against the ancestral sequence was reduced by 3.7-fold in convalescent and 1.7-fold in naive individuals. Also, the mean NT_{50} against all VoC were significantly reduced at 8 months postvaccination (range 2- to 3.7-fold; $P = .0034$ to $P < .0001$). Specifically, for the dominant SARS-CoV-2 delta VoC, NT_{50} at 8 months after vaccination were 839 and 118, respectively, for COVID-19–convalescent and COVID-19–naive individuals. The decline of NT_{50} titer against delta was similar in both groups (2.3- vs 2.9-fold; $P > .99$ and $P = .08$, not significant, respectively); however, after 8 months, neutralizing activity against delta was not detectable in 4/21 (19%) of the COVID-19–naive vaccinated group.

DISCUSSION

The level of neutralizing antibodies is the main surrogate marker for efficacy in most viral vaccines [7]. In COVID-19 the correlates of protection for both infection and severe disease are currently unknown. Considering the enormous heterogenicity in the clinical expression of COVID-19, this is particularly relevant. A certain level of neutralizing antibodies at the upper respiratory tract mucosa could be protective for infection, as has been demonstrated in animal models [8] and clinical studies [9]. If SARS-CoV-2 infection takes place, memory B- and T-cell responses are thought to play an important role because severe COVID-19 develops within a time frame that allows their activation and effector functions [8, 10]. In real-world experiences, it is clear that vaccine efficacy against severe disease remains relatively stable at least up to several months postvaccination but full protection against infection exhibits a continuous decline [10]. This waning effect of vaccine protection against infection is especially relevant in the midst of the current surge of the delta variant, which has shown high transmissibility that appears to be related to a faster spike-mediated cell fusion upon ACE2 interaction [11].

In our study we detected a significant reduction of RBD binding antibodies from month 2, at the presumably higher level of response, to month 8 after BNT162b2 vaccination: 3.7- and 1.7-fold in COVID-19–convalescent and COVID-19–naive individuals, respectively. This is reflected in the reduction of neutralizing activity against the VoC tested, ranging from 2- to 3.7-fold during the follow-up period. With the current surge, the delta VoC is becoming dominant in most areas, and so it is important to follow the evolution of the neutralizing response against this highly transmissible variant. Our data demonstrated that there was an overall 6.3-fold decline in the neutralizing activity of the response induced by BNT162b2 vaccine in naive individuals, including a reduced response to delta as compared with Wuhan-Hu-1 (2.2-fold) and a waning effect over time (2.9-fold). This decline resulted in a mean NT_{50} titer of 118 (GMT) and significant proportion (19%) of naive individuals without detectable neutralization activity after 8 months. A similar decline was experienced in the convalescent vaccinated group; however, the median titer level was much higher (839 GMT) and all individuals had detectable neutralizing antibody level. Similar results on the evolution of neutralizing response have been reported for different groups and ages [12, 13].

Although breakthrough infection in vaccinated individuals is presumably a multifactorial event, low levels of neutralizing response against the delta variant in serum, and likely in mucosa, could be a relevant factor for infection of this variant that is highly adapted to human transmission [14]. Most breakthrough SARS-CoV-2 infections appear not to result in clinically severe disease but can maintain chains of transmission among vaccinated and unvaccinated contacts [15]. This might be especially important in areas with low vaccine coverage.

Finally, the identification of surrogate biomarkers for vaccine protection is much needed and precise follow-up on neutralizing activity evolution in different groups connected with clinical data could be helpful to establish correlates of protection. Surveillance of the evolution of the breadth of neutralizing response against VoC could inform decisions for boosting strategies and should be taken into account to develop adapted immunogens against VoC with high immune escape potential.

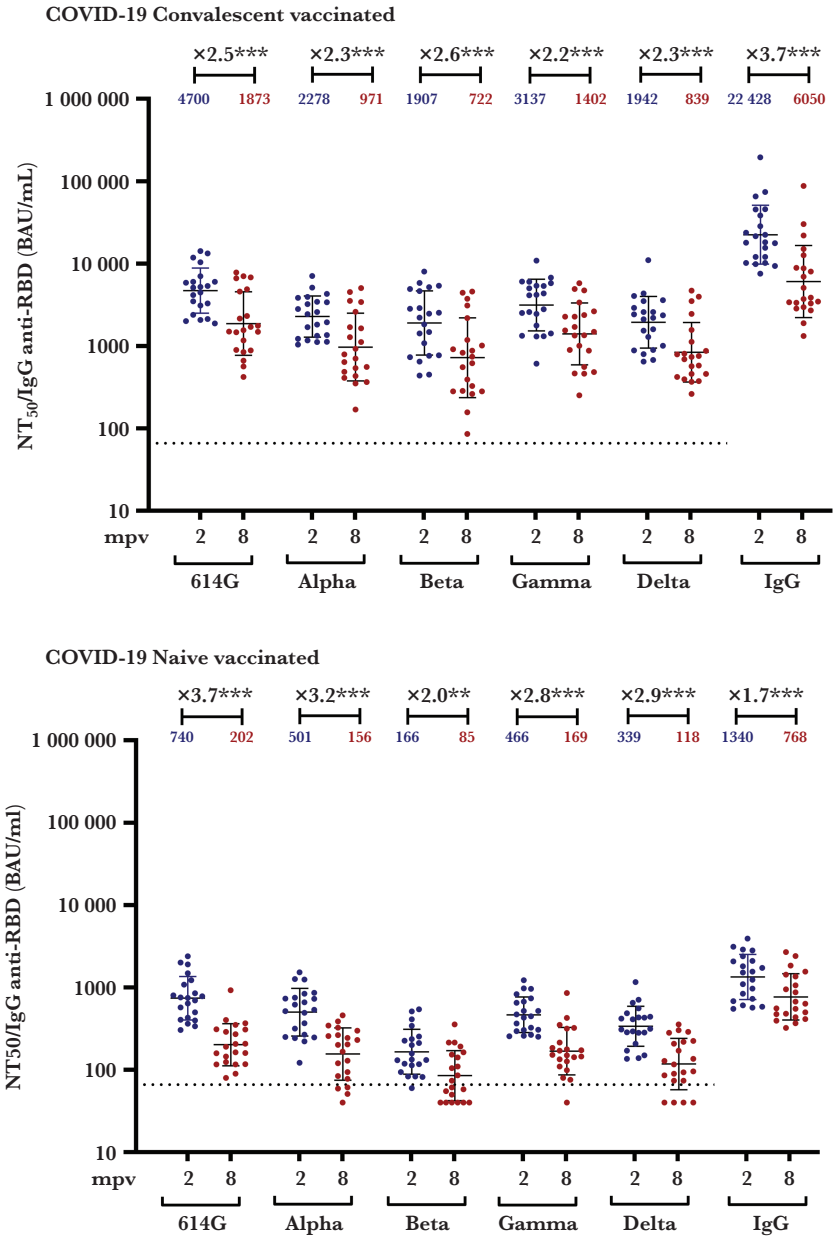

Figure 1. SARS-CoV-2 RBD-specific IgG BAU and serum NT_{50} against SARS-CoV-2 VoC: reference 614G, alpha, beta, gamma, and delta. COVID-19–convalescent vaccinated (n = 21) and COVID-19–naive vaccinated (n = 21) individuals were tested at 2 and 8 months after BNT162b2 vaccination. Individual NT_{50} and anti-RBD IgG values are presented as scatter dot plot (blue, 2 mpv; red, 8 mpv). Solid lines and blue and red numbers are geometric means. Error bars correspond to SEM. Dashed line marks the cutoff titer for neutralization assay (NT_{50} 1/66). NT_{50} was calculated from individual results obtained in triplicate using a nonlinear regression model fit with settings for log inhibitor versus normalized response curves by GraphPad Prism version 8. RBD-specific IgG titers are presented as BAU/mL. Fold decrease in NT_{50} and anti-RBD at 2 and 8 mpv, with statistical significance, are indicated above scatter dot results for each variant and anti-RBD IgG. Statistical analysis was performed by Wilcoxon matched-pair signed-rank test in GraphPad Prism version 8. *$P < .05$, **$P < .01$, ***$P < .001$. Abbreviations: BAU, binding antibody unit; COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; mpv, months postvaccination; NT_{50}, 50% neutralizing titer; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VoC, variant of concern.

Notes

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