Neutralizing response against SARS-CoV-2 variants 8 months after BNT162b2 vaccination in naïve and COVID-19 convalescent individuals

Joanna Luczkowiak1, Nuria Labiod1, Gonzalo Rivas2, Marta Rolo2, Fátima Lasala1, Jaime Lora-Tamayo3, Mikel Mancheno-Losa3, David Rial-Crestelo3, Alfredo Pérez-Rivilla2,4, María Dolores Folgueira1,2,4, Rafael Delgado*1,2,4

1-Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid, Spain
2-Department of Microbiology. Hospital Universitario 12 de Octubre, Madrid, Spain
3-Department of Internal Medicine. Hospital Universitario 12 de Octubre, Madrid, Spain
4-School of Medicine. Universidad Complutense. Madrid, Spain

© The Author(s) 2021. Published by Oxford University Press for the Infectious Diseases Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
*Corresponding author: Dr. Rafael Delgado, Servicio de Microbiología, Hospital Universitario 12 de Octubre, Avenida de Córdoba sn, Madrid 28041 Spain, Phone +34 669580985, e-mail: rafael.delgado@salud.madrid.org

Brief Summary:

Eight months after BNT162b2 vaccination neutralizing activity declined 2-3.7-fold for all VoC and 19% of naïve individuals lacked neutralizing activity against the SARS-CoV-2 delta variant. In convalescents vaccinated the high initial humoral response resulted in detectable neutralization against all VoC.
Footnote page:

Conflict of Interest Statement:

All authors declare not having conflicts of interests related to this work

Funding Statement:

Research in RD lab is supported by grants from the Instituto de Investigación Carlos III, ISCIII, (FIS PI1801007 and PI2100989), by the European Commission Horizon 2020 Framework Programme: Project VIRUSCAN FETPROACT-2016: 731868, Horizon Europe Framework programme: Project EPIC-CROWN-2 ID: 101046084 and by Fundación Caixa-Health Research (Project StopEbola HR18-00469). M.M-L holds a clinical research contract Rio Hortega (CM19/00226) from the Instituto de Salud Carlos III, ISCIII (Spanish Ministry of Science, Innovation and Universities).
Corresponding Author:

Dr. Rafael Delgado, Servicio de Microbiología, Hospital Universitario 12 de Octubre, Avenida de Córdoba sn, Madrid 28041 Spain, Phone +34 669580985, e-mail:

rafael.delgado@salud.madrid.org
Abstract

We have investigated the evolution of the neutralizing response against SARS-CoV-2 variants at 8 months after Pfizer-BNT162b2 vaccination in COVID-19 naïve (n=21) and COVID-19 convalescent (n=21) individuals. Neutralizing levels declined for all variants (range 2–3.7-fold). Eight months after vaccination a significant proportion (4/21) of naïve 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
Background:

Vaccination against SARS-CoV-2 is highly protective against severe forms of 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 have included COVID-19 naïve \((n=21)\) and COVID-19 convalescent \((n=21)\) healthcare workers (HCW) from the Hospital Universitario 12 de Octubre in Madrid, Spain. The two groups were part of a follow-up study (Solidarity II cohort, IRB approval ref CElm 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 naïve 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 two doses of the Pfizer-BNT162b2 vaccine 21 days apart(4). Blood samples were obtained at 61 days (range 42-77) and 242 days (range 238-252) after the first dose in the convalescent group and at 67 days (range 49-97) and 241 (range 228-252) in the naïve group.

ELISA anti-RBD IgG

Anti-RBD IgG (AbRBD) titers were determined by an electrochemiluminescence commercial assay (Elecsys Anti-SARS-CoV-2, Roche Diagnostics, Basel, Switzerland) and were converted to WHO 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 rVSV-luc (PSV) system. PSV were 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 (CNB-CSIC, 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 Fisher Scientific GENEART GmbH, Regensburg, Germany). Serum samples were heat-inactivated at 56 °C for 30 min and tested at dilutions 1:80, 160, 320, 640, 1280, 2560, 5120. Pseudotyped viruses were normalized for infectivity to a MOI of 0.5-1 and incubated with the dilutions of serum samples at 37º C for 1 h in 96-well plates. After the incubation time, 2 x 10⁴ Vero E6 cells were seeded onto the virus-plasma mixture and incubated in a 37ºC for 24h. Cells were then lysed and assayed for luciferase expression. Neutralizing titer 50% (NT50) was calculated using a nonlinear regression model fit with settings for log inhibitor versus normalized response curves, in GraphPad Prism v8 and is expressed as the reciprocal dilution. Means of AbRBD and NT50 titers were calculated as geometric mean titers (GMT). Statistical significance among titers was calculated using Wilcoxon matched-paired signed rank test or Multiple Comparisons One-way ANOVA Friedman test with Dunn’s correction by GraphPad Prism v8.
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 naïve at 2 month post-vaccination (16.7- and 6.3-fold in AbRBD and NT50 against the ancestral sequence respectively (both p<0.0001). The beta VoC exhibited the highest neutralizing reduction: 2.5-fold in “convalescents” and 4.5-fold in “naïve”.

Eight months after the first dose, AbRBD against the ancestral sequence was reduced by 3.7-fold in “convalescent” and 1.7-fold in “naïve”. Also the mean NT50 against all VoC was significantly reduced at 8 months post-vaccination (range 2-3.7-fold) (p range: p=0.0034 to p<0.0001). Specifically, for the dominant SARS-CoV-2 delta VoC, NT50 at 8 months after vaccination was 839 and 118 respectively for COVID-19 convalescent and COVID-19 naïve individuals. The decline of NT50 titre against delta was similar in both groups (2.3- vs 2.9-fold) (p>0.99 and p=0.08, not significant, respectively); however, after 8 months neutralizing activity against delta was not detectable in 4/21 (19%) of the COVID-19 naïve vaccinated group.
Discussion:

The level of neutralizing antibodies is the main surrogate marker for efficacy in most viral vaccines(7). In COVID-19 it is currently unknown the correlates of protection for both infection and severe disease. 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 since 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 post vaccination 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 have detected a significant reduction of RBD binding antibodies from month 2, at the presumably higher level of response, to month 8 post BNT162b2
vaccination: 3.7 and 1.7-fold in COVID-19 convalescent and COVID-19 naïve 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. The current surge of the delta VoC is becoming dominant in most of the areas, 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 naïve individuals considering the reduced response of delta as compared with the control (2.2-fold) and the time waning effect (2.9-fold). This decline results in a mean NT50 titre of 118 (GMT) and significant proportion (19%) of naïve individuals without detectable neutralization activity after 8 months. A similar decline is experienced in the convalescent vaccinated group; however the median titre level is much higher (839 GMT), and all individuals had detectable neutralizing antibody level. Similar results on evolution of neutralizing response have been reported for different groups and ages(12, 13).

Although breakthrough infection in vaccinated 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 highly adapted to human transmission(14). Most breakthrough SARS-CoV-2 infections appear not to result in
clinical 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 is to be taken into account to develop adapted immunogens against VoC with high immune escape potential.
Acknowledgments:

We are greatly thankful to the group of participants health workers for their generous contribution to the study along with the WHO Solidarity2 project for supporting and maintaining a great collaborative network.
References

1. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med. 2020;383(25):2439-50.

2. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020;383(20):1920-31.

3. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. N Engl J Med. 2021;385(16):1474-84.

4. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-15.

5. Whitt MA. Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. J Virol Methods. 2010;169(2):365-74.
6. Luczkowiak J, Labiod N, Rivas G, Rolo M, Lasala F, Lora-Tamayo J, et al. Prime-Boost Vaccination With BNT162b2 Induces High Neutralizing Activity Against SARS-CoV-2 Variants in Naïve and COVID-19-Convalescent Individuals. Open Forum Infect. Dis. 2021; 8(10). DOI: 10.1093/ofid/ofab468.

7. Plotkin SA. Correlates of protection induced by vaccination. Clin. Vaccine Immunol. 2010;17(7):1055-65.

8. Gagne M, Corbett KS, Flynn BJ, Foulds KE, Wagner DA, Andrew SF, et al. Protection from SARS-CoV-2 Delta one year after mRNA-1273 vaccination in nonhuman primates is coincident with an anamnestic antibody response in the lower airway. bioRxiv. 2021:2021.10.23.465542.

9. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 2021;184(4):861-80.

10. Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. N Engl J Med. 2021. 385:e85

11. Zhang J, Xiao T, Cai Y, Lavine Christy L, Peng H, Zhu H, et al. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science. 2021. 0(0):eabj9463.
12. Tober-Lau P, Schwarz T, Vanshylla K, Hillus D, Gruell H, Group ECS, et al. Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. Lancet Respir Med. 2021;9(11):e104–e5.

13. Cassaniti I, Bergami F, Percivalle E, Gabanti E, Sammartino JC, Ferrari A, et al. Humoral and cell-mediated response against SARS-CoV-2 variants elicited by mRNA vaccine BNT162b2 in healthcare workers: a longitudinal observational study. Clin. Microbiol. Infect. 2021. Sep 25;S1198-743X(21)00536-X.

14. Rosenberg ES, Dorabawila V, Easton D, Bauer UE, Kumar J, Hoen R, et al. COVID-19 Vaccine Effectiveness by Product and Timing in New York State. medRxiv. 2021:2021.10.08.21264595.

15. Singanayagam A, Hakki S, Dunning J, Madon KJ, Crone MA, Koycheva A, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. Lancet Infect. Dis. 2021. DOI:https://doi.org/10.1016/S1473-3099(21)00648-4
Figure 1 Legend:

Figure 1: SARS-CoV-2 RBD-specific IgG Binding antibody units (BAU) and serum neutralizing activity (NT50) against SARS-CoV-2 VoC: reference 614G, Alpha, Beta, Gamma and Delta. COVID-19 convalescent vaccinated (n=21) and COVID-19 naïve vaccinated (n=21) individuals were tested at 2- and 8-months post BNT162b2 vaccination (mpv). Individual NT50 and anti-RBD IgG values are presented as scatter dot plot (2 mpv in blue and 8 mpv in red). Solid lines and numbers correspond to geometric mean. Dashed line marks the cut-off titre for neutralization assay (NT50 1/66). NT50 was calculated from individual results obtained by triplicates using a nonlinear regression model fit with settings for log inhibitor versus normalized response curves by GraphPad Prism v8. RBD-specific IgG titres are presented as BAU/ml. Fold decrease in NT50 and anti-RBD at 2 and 8 mpv, together 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 v8. *p<0.05; **p<0.01; ***p<0.001. Abbreviations: mpv, months postvaccination, NT50, neutralizing titer 50; IgG, immunoglobulin G; BAU/ml, binding antibody units per ml.
Figure 1

COVID-19 convalescent vaccinated

COVID-19 naïve vaccinated