Neutralization heterogeneity of United Kingdom and South-African SARS-CoV-2 variants in BNT162b2-vaccinated or convalescent COVID-19 healthcare workers

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ABSTRACT

There are concerns about neutralizing antibodies (NAbs) potency against SARS-CoV-2 variants. Despite decreased NAb titers elicited by BNT162b2-vaccine against VOC202012/01 and 501Y.V2 (SA) strains, 28/29 healthcare workers (HCW) had a NAb titer ≥ 1:10. In contrast, six months after COVID-19 mild forms, only 9/15 (60%) of HCW displayed detectable NAbs against SA strain.

INTRODUCTION

In the gene encoding the Spike (S) protein of SARS-CoV-2, various mutations have been reported[1,2] and recently, the United Kingdom (UK) and South Africa (SA) have faced a rapid increase in COVID-19 cases mediated by the emergence of new variants (VOC-202012/01 for UK and 501Y.V2 for SA)[3,4]. The spreading of these variants has increased rapidly in other countries and recent observations suggests that they are significantly more transmissible than previously circulating variants. It is still not fully known if the pathogenicity is either increased, although some elements have been recently released with likely enhanced disease severity for the UK strain[5].

These variants harbor a specific pattern of deletion and mutations including amino-acid replacements at key sites in the S Receptor Binding Domain (RBD) (K417N, E484K, N501Y for the SA strain and only N501Y for the UK strain) and in the N-terminal domain (Δ69/70 and ΔY144 deletions for the UK strain and L18F, D80A, D215G and Δ242-244 for the SA strain). In the era of the COVID-19 vaccination, the question remained whether these variants could escape the neutralizing response elicited by mRNA-vaccine. Two recent studies performed on engineered SARS-CoV-2 viruses containing only some mutations from the newly emerged UK and SA variants showed weaker neutralization capacity of vaccine-elicited sera[6,7]. Another study tested SARS-CoV-2-S pseudoviruses bearing either the Wuhan reference strain or the UK spike protein with BNT162b2 vaccine-elicited sera showed a slightly reduced but overall largely preserved neutralizing titers against the UK pseudovirus[8]. However, none of these studies was performed on clinical isolates harboring the full genomic mutations background of UK and SA strains. Thus, the question remained whether a replicating virus with the full set of S mutations, which may potentially interfere with antibody binding would be neutralized efficiently by convalescent COVID-19 or BNT162b2-immune sera, especially in the healthcare workers (HCW), a particularly exposed population to SARS-CoV-2 infection.

To answer this question, we performed a virus neutralization test (VNT), with a strict 100% inhibition criterion, on sera from HCW with either previous mild forms of COVID-19 or BNT162b2 immunization using three clinical isolates of SARS-CoV-2 variants: a D614G strain (D614G) which became the dominant form of the virus circulating globally in the second part of 2020[2], a UK strain (UK, lineage B.1.1.7) and a SA strain (SA, lineage B.1.351).

MATERIALS AND METHODS

Study population and serum specimen

Convalescent sera were recovered six months after symptom's onset from symptomatic HCW with a positive RT-PCR result. BNT162b2-vaccine elicited sera were recovered three weeks after the first injection and seven days after the booster immunization. This retrospective study was carried out in accordance with the Declaration of Helsinki without addition to standard of care procedures. Data collection were declared to the Sorbonne Université Data Protection Committee under number 2020-025. Written informed consent for participation in this study was obtained from all participants.

Virus neutralization test

The neutralizing activity of the various serum specimen was assessed with a whole virus replication assay as previously described (*9*) using three SARS-CoV-2 clinical isolates D614G, UK and SA (GenBank accession number MW322968, MW633280 and MW580244 respectively). Microscopy examination was performed on day 4 to assess the cytopathic effect (CPE). Neutralizing antibody (NAb) titers are expressed as the highest serum dilution displaying 100% (NT_{100}), 90% (NT_{90}) or 50% (NT_{50}) inhibition of the CPE. A same known positive control serum was added to each experiment to assess the repeatability.

Statistical analysis

NT_{50} or NT_{90} were inferred by non-linear regression using a four-parameter variable slope model using GraphPad Prism 8.0.2 software. Geometric mean titer (GMT) with 95% confidence interval (95%CI) were calculated for NT_{100}, NT_{50} and NT_{90} (Figure 1 and Table S1). Difference in distribution of NT_{100} between UK strain or SA strain with the D614G strain was performed with a two-tailed Mann-Whitney-U test. A probability value of $p<0.05$ was considered statistically significant. No statistical comparisons were made between post vaccines and mild COVID-19 groups.

RESULTS

We studied two sets of serum samples from HCW: a convalescent group of 15 participants with SARS-CoV-2 proven infection on March 2020 and a vaccinated group of 29 participants without history of clinical COVID-19. The median [IQR] age was 50 [32 – 66] years and 40% (6/15) were male for the convalescent group. The median age was 55 [38 – 65] and 31% (9/29) were male for the vaccinated group. Convalescent sera were collected 6 months after the symptom's onset (184 [182 – 189] days). Three weeks after the first injection of the BNT162b2 vaccine, 52% (13/25) of HCW harbored $NT_{100} \geq 1:5$ against the D614G strain, 24% (6/25) were neutralizing against the UK strain and only two (8%) had detectable NAbs against the SA strain (Figure 1A). Seven days after the booster immunization, all but one HCW displayed ~~neutralizing activity~~ NT_{100} against the three SARS-CoV-2 clinical strains with a GMT of 117.3 (95%CI, 90.4 to 152.0) against the D614G strain, 45.1 (95%CI, 34.3 to 59.3) against the UK strain and 22.9 (95%CI, 16.6 to 31.6) against the SA strain. The NT_{100} against UK and SA strains were significantly reduced compared to NT_{100} against the D614G strain 7 days after the second injection of BNT162b2 vaccine (respectively, $p < 0.0001$ and $p < 0.0001$) (Figure 1B). Six months after the symptom's onset, all the 15 HCW of the convalescent group harbored NT_{100} against the D614G strain (GMT of 21.0; 95%CI, 11.8 to 37.1) and the UK strain (GMT

of 14.5; 95%CI, 8.8 to 23.9) without statistical difference between the respective NT_{100} ($p = 0.40$). However, only 60% (9/15) serum samples of these HCW displayed a neutralizing activity against the SA strain with a NT_{100} GMT of 3.3 (95%CI, 1.8 to 6.1; $p < 0.0001$) (Figure 1C).

DISCUSSION

In this work we assessed the neutralizing activity of sera from 15 convalescent COVID-19 or 29 BNT162b2-vaccinated HCW against the two rapidly spreading SARS-CoV-2 variants of concern VOC202012/01 and 501Y.V2 and the globally circulating variant D614G using a VNT with whole replicating clinical strains. Based on a very strict criterion of 100% inhibition of CPE to determine NT_{100}, we show that, three weeks after a single dose of BNT162b2, these NT_{100} remain inexistent or low among HCW especially against the UK and SA variants and could questioned the extend of the dosing interval of BNT162b2 in some countries in order to vaccinate as many people as possible. This observation was confirmed with less strict/restrictive criterions of NT_{90} or NT_{50} (Table S1). However, we were not able to follow participants more than three weeks after the first injection because all of them received a second dose of BNT162b2 according to the French guidelines. Nevertheless, seven days after the booster immunization all but one vaccinated HCW develop NAbs against the three strains with a highest neutralizing activity against the strain closely related to the Wuhan ancestral strain, the D614G strain. Despite a 2.60-fold reduction of NT_{100} GMT against the UK and a 5.12-fold reduction against the SA strains in comparison with the D614G most of the participants have displayed a neutralizing activity $\geq$ 1:10 which could be at least indicative of a potential protection against severe COVID-19 even with these variants. Although the correlates of protection are not already known, there is probably a certain degree of protection before the NAbs are detectable.

Based on NT_{100}, NT_{90} and NT_{50} values, we also demonstrate a lack of serum neutralizing activity against SA strain in up to 40% of HCW recovered from mild form of COVID-19 six

144 months after the symptom's onset associated with a 6.32 to 7.17-fold reduction of GMT in the
145 HCW with detectable NAbs. This finding, and the recent report describing a severe case of
146 reinfection by the SA variant four months after a first COVID-19 infection[9], highlights the
147 need of vaccination even in people who had recovered from a previous COVID-19, especially
148 during the increased circulation of the SARS-CoV-2 variants. Nevertheless, correlates of
149 immunity to the SARS-CoV-2 are not well defined, only few studies have tried to assess these
150 correlates in other human coronaviruses with experimental challenges on volunteers. They
151 showed an association between serum NAb titers pre-exposure and viral excretion[10]. Further
152 studies are required to determine the SARS-CoV-2 correlates of vaccine-induced protection
153 based on NAb and T cell responses. A limitation of our work is that we were not able to assess
154 potential cellular response differences against the three strains in the vaccinated or convalescent
155 groups although it has been described generation of a robust CD4+ and CD8+ responses against
156 the Wuhan ancestral strain[11]. The long-term evaluation regarding the lasting of NAb induced
157 by vaccination is needed to assess the durability of protection against SARS-CoV-2 variants.

158

159 **CONCLUSION**

160 In conclusion, in BNT162b2-vaccinated participants with two dose regimen, despite
161 heterogeneity neutralizing capacity against the three SARS-CoV-2 variants, most of the sera
162 harbored at least a NAb titer ≥ 1:10. Although immune protection correlates need to be defined,
163 our findings suggests a certain humoral protection activity either on UK or SA variants after
164 two doses of mRNA-vaccine. We also show that six months after SARS-CoV-2 infection
165 leading to mild forms of COVID-19, an important proportion of HCW displayed no neutralizing
166 activity against SA strain. This result supports a strong recommendation for SARS-CoV-2
167 vaccination of previously infected subjects.

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Competing interests: Authors declare that they have no competing interests.

REFERENCES

1. A. A. Dawood, Mutated COVID-19 may foretell a great risk for mankind in the future. New Microbes New Infect. 35, 100673 (2020).

2. B. Korber, W. M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, N. Hengartner, E. E. Giorgi, T. Bhattacharya, B. Foley, K. M. Hastie, M. D. Parker, D. G. Partridge, C. M. Evans, T. M. Freeman, T. I. de Silva, Sheffield COVID-19 Genomics Group, C. McDanal, L. G. Perez, H. Tang, A. Moon-Walker, S. P. Whelan, C. C. LaBranche, E. O. Saphire, D. C. Montefiori, Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 182, 812-827.e19 (2020).

3. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa | medRxiv, (available at https://www.medrxiv.org/content/10.1101/2020.12.21.20248640v1).

4. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology. Virological (2020), (available at https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563).

5. G. Iacobucci, Covid-19: New UK variant may be linked to increased death rate, early data indicate. BMJ. 372, n230 (2021).

6. X. Xie, Y. Liu, J. Liu, X. Zhang, J. Zou, C. R. Fontes-Garfias, H. Xia, K. A. Swanson, M. Cutler, D. Cooper, V. D. Menachery, S. C. Weaver, P. R. Dormitzer, P.-Y. Shi, Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. Nature Medicine, 1–2 (2021).

7. Serum Neutralizing Activity Elicited by mRNA-1273 Vaccine — Preliminary Report | NEJM, (available at https://www.nejm.org/doi/full/10.1056/NEJMc2102179).

8. A. Muik, A.-K. Wallisch, B. Sänger, K. A. Swanson, J. Mühl, W. Chen, H. Cai, D. Maurus, R. Sarkar, Ö. Türeci, P. R. Dormitzer, U. Şahin, Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine–elicited human sera. Science (2021), doi:10.1126/science.abg6105.

9. S. Marot, I. Malet, V. Leducq, K. Zafilaza, D. Sterlin, D. Planas, A. Gothland, A. Jary, K. Dorgham, T. Bruel, S. Burrel, D. Boutolleau, O. Schwartz, G. Gorochov, V. Calvez, A.-G. Marcelin, Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. Nature Communications. 12, 844 (2021).

10. N. Zucman, F. Uhel, D. Descamps, D. Roux, J.-D. Ricard, Severe reinfection with South African SARS-CoV-2 variant 501Y.V2: A case report. Clin Infect Dis (2021), doi:10.1093/cid/ciab129.

11. A. T. Huang, B. Garcia-Carreras, M. D. T. Hitchings, B. Yang, L. C. Katzelnick, S. M. Rattigan, B. A. Borgert, C. A. Moreno, B. D. Solomon, L. Trimmer-Smith, V. Etienne, I. Rodriguez-Barraquer, J. Lessler, H. Salje, D. S. Burke, A. Wesolowski, D. A. T. Cummings, A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. Nat Commun. 11, 4704 (2020).

12. U. Sahin, A. Muik, E. Derhovanessian, I. Vogler, L. M. Kranz, M. Vormehr, A. Baum, K. Pascal, J. Quandt, D. Maurus, S. Brachtendorf, V. Lörks, J. Sikorski, R. Hilker, D. Becker, A.-K. Eller, J. Grützner, C. Boesler, C. Rosenbaum, M.-C. Kühnle, U. Luxemburger, A. Kemmer-Brück, D. Langer, M. Bexon, S. Bolte, K. Karikó, T. Palanche, B. Fischer, A. Schultz, P.-Y. Shi, C. Fontes-Garfias, J. L. Perez, K. A. Swanson, J. Loschko, I. L. Scully, M. Cutler, W. Kalina, C. A. Kyratsous, D. Cooper, P. R. Dormitzer, K. U. Jansen, Ö. Türeci,

COVID-19 vaccine BNT162b1 elicits human antibody and T H 1 T cell responses. Nature. 586, 594–599 (2020).

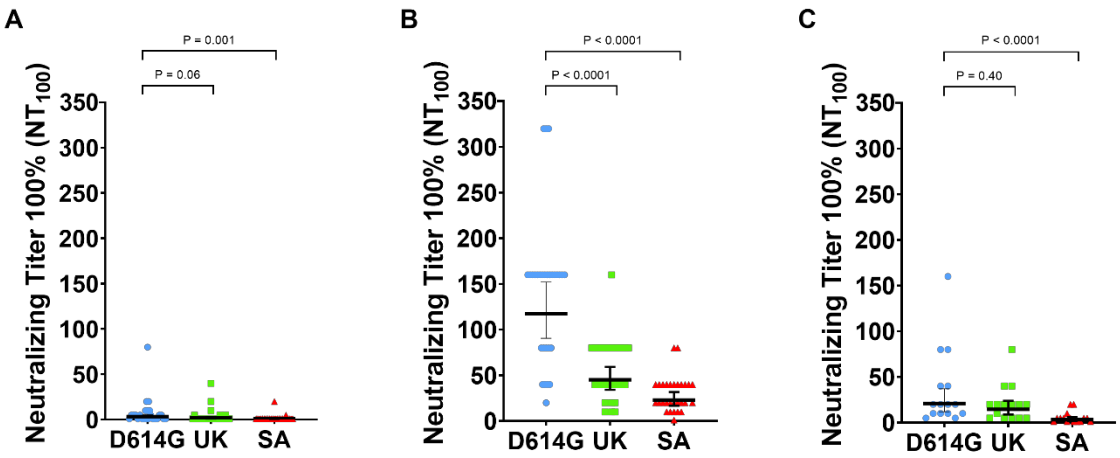

Fig. 1. Neutralizing antibody (NAb) titer with 100% inhibition (NT_{100}) against clinical strains of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants of 29 BNT162b2-vaccine elicited sera and 15 convalescent sera recovered from healthcare workers (HCW). (A) NT_{100} against the three clinical isolates of BNT162b2-vaccine elicited HCW sera recovered three weeks after first injection. (B) NT_{100} against the three clinical isolates of BNT162b2-vaccine elicited HCW sera recovered seven days after second injection. (C) NT_{100} against the three clinical isolates of convalescent COVID-19 HCW sera recovered 6 months after the symptom's onset. NT_{100} against D614G strain are in blue dot, NT_{100} against UK strain are in green square and NAb titer against SA strain are in red triangle. Black horizontal lines indicate geometric median titer (GMT) of NT_{100}. Whiskers indicate 95% confidence interval. Two-tailed P values were determined using the Mann-Whitney test and are reported on each panel.

Table S1. Geometric median titer (GMT) of neutralizing antibody (NAb) with 50% (NT_{50}) or 90% (NT_{90}) inhibition against clinical strains of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants of 29 BNT162b2-vaccine elicited sera and 15 convalescent sera recovered from healthcare workers (HCW). 95% confidence interval were written between parenthese.

	D614G strain		UK strain		SA strain		GMT ratio D614G/UK		GMT ratio D614G/SA	
	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90
BNT162b2 - 1st injection	5.38 (3.0 to 9.6)	4.90 (2.8 to 8.7)	3.46 (2.0 to 5.7)	2.89 (1.8 to 4.7)	1.47 (1.0 to 2.2)	1.39 (1.0 to 2.0)	1.56	1.70	3.66	3.53
BNT1622 - 2nd injection	174.2 (133.5 to 227.2)	157.9 (122.4 to 203.8)	72.34 (52.8 to 99.1)	55.89 (39.2 to 79.6)	40.55 (28.5 to 57.7)	31.99 (22.4 to 45.8)	2.41	2.83	4.30	4.94
Convalescent	35.40 (20.2 to 62.1)	30.88 (17.1 to 55.7)	28.11 (16.5 to 47.8)	20.04 (12.1 to 33.2)	4.94 (2.4 to 10.1)	4.43 (2.3 to 8.5)	1.26	1.54	7.17	6.97