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

Stéphane Marot1*, Isabelle Malet1, Valentin Leducq1, Basma Abdi1, Elisa Teyssou1, Cathia Soulie1, Marc Wirden1, Christophe Rodriguez2,3, Slim Fourati2,3, Jean-Michel Pawlotsky2,3, David Boutolleau1, Sonia Burrel1, Vincent Calvez1, Anne-Geneviève Marcelin1, Aude Jary1

1Sorbonne Université, INSERM, Institut Pierre Louis d’Épidémiologie et de Santé Publique (iPLESP), Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié Salpêtrière Hospital, Department of Virology, Paris, France.

2Department of Virology, Hôpitaux Universitaires Henri Mondor, AP-HP, Créteil, France.

3Team “Viruses, Hepatology, Cancer”, Institut Mondor de Recherche Biomédicale, INSERM U955, Université Paris-Est, Créteil, France.

*Corresponding author. Stephane Marot, Department of Virology, Pitié Salpêtrière Hospital, AP-HP, CERVI, 83 Boulevard de l’Hôpital, 75013, Paris, France. Email: stephanesylvain.marot@aphp.fr
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 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 501Y.V2 strain.

Keywords: COVID-19, Vaccine, Neutralizing antibodies, SARS-CoV-2 variants
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<sub>100</sub>), 90% (NT<sub>90</sub>) or 50% (NT<sub>50</sub>) inhibition of the CPE. A same known positive control serum was added to each experiment to assess the repeatability.

**Statistical analysis**

NT<sub>50</sub> or NT<sub>90</sub> 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<sub>100</sub>, NT<sub>50</sub> and NT<sub>90</sub> (Figure 1 and Table S1). Difference in distribution of NT<sub>100</sub> 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<sub>100</sub> ≥ 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 NT<sub>100</sub> 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<sub>100</sub> 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 \( \text{NT}_{100} \), \( \text{NT}_{90} \) and \( \text{NT}_{50} \) 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 months after the symptom’s onset associated with a 6.32 to 7.17-fold reduction of GMT in the HCW with detectable NAbs. This finding, and the recent report describing a severe case of reinfection by the SA variant four months after a first COVID-19 infection\[9\], highlights the need of vaccination even in people who had recovered from a previous COVID-19, especially during the increased circulation of the SARS-CoV-2 variants. It is worth noting that three convalescent HCW were vaccinated with one injection of BNT162B2, which elicited a 16 to 128-fold change of \( \text{NT}_{100} \) against D614G and UK strains and a 32 to 160-fold change against SA strain. Nevertheless, correlates of immunity to the SARS-CoV-2 are not well defined, only few studies have tried to assess these correlates in other human coronaviruses with experimental challenges on volunteers. They showed an association between serum NAb titers pre-exposure and viral excretion\[10\]. Further studies are required to determine the SARS-CoV-2 correlates of vaccine-induced protection based on NAb and T cell responses. A limitation of our work is that we were not able to assess potential cellular response differences against the three strains in the vaccinated or convalescent groups although it has been described generation of a robust CD4+ and CD8+ responses against the Wuhan ancestral strain\[11\]. The long-term evaluation regarding the lasting of NAb induced by vaccination is needed to assess the durability of protection against SARS-CoV-2 variants.
CONCLUSION

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

Acknowledgments: We thank all the members of the Pitié-Salpêtrière Virology Department for their active collaboration and Vanessa Demontant, Elisabeth Trawinski, Melissa N’Debi and Guillaume Gricourt for helpful technical assistance in the full genome sequencing of the UK and SA strains.

Funding: This work was supported by the Agence Nationale de la Recherche sur le SIDA et les Maladies Infectieuses Emergentes (ANRS MIE), AC43 Medical Virology and the SARS-CoV-2 Program of the Faculty of Medicine of Sorbonne Université.

Competing interests: SF reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Abbvie and Abbott Diagnostics and has served on a DSMB/Advisory Board for MSD. All other authors declare that they have no competing interests.
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Fig. 1. Neutralizing antibody (NAb) titer with 100% inhibition ($\text{NT}_{100}$) 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) $\text{NT}_{100}$ against the three clinical isolates of BNT162b2-vaccine elicited HCW sera recovered three weeks after first injection. (B) $\text{NT}_{100}$ against the three clinical isolates of BNT162b2-vaccine elicited HCW sera recovered seven days after second injection. (C) $\text{NT}_{100}$ against the three clinical isolates of convalescent COVID-19 HCW sera recovered 6 months after the symptom’s onset. $\text{NT}_{100}$ against D614G strain are in blue dot, $\text{NT}_{100}$ 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 $\text{NT}_{100}$. Whiskers indicate 95% confidence interval. Two-tailed P values were determined using the Mann-Whitney test and are reported on each panel.
