A booster dose of either BNT162b2 or mRNA-1273 mRNA vaccines induces a robust recall of anti-SARS-CoV-2 spike protein IgG antibodies in saliva

Frederick E. Turner[1], Robert Brown[1], Prithivi Chellamuthu[1], Edwyn Saavedra[1], Nicholas Denny[1], Marilisa Santacruz[1], Aaron N. Angel[1], Melanie A. MacMullan[1], Maria Shacreaw[1], Lauren Lopez[1], Victoria Hess[1], Noah Kojima[1], Jeffery D. Klausner[1], Matthew Brobeck[1], Vladimir I. Slepnev[1], and Albina Ibrayeva[1]

[1]Curative Inc

March 3, 2023

Abstract

Saliva specimens offer practical advantages over serum specimens for studying SARS-CoV-2 immunity following natural infections or vaccination. Salivary anti-spike (S)-protein immunoglobulin G (IgG) antibody titers are quantifiable by ELISA with high sensitivity and specificity and robustly correlated with serum titers. Our longitudinal prospective study enrolled participants who received two-dose regimen vaccination with either mRNA-1273 or BNT162b2 vaccines, and salivary anti-S-protein IgG titers were measured at intervals for its duration. Subsequently, participants received homologous mRNA-1273 (n=28) and BNT162b2 (n=29) booster vaccines and enrolled in the booster study. Participants performed self-collection of saliva specimens with the OraSure ® device at predetermined time intervals for each cohort. Salivary anti-S-protein IgG titers varied between participants following the second dose; titers waned from their peak 9 to 150-fold in the mRNA-1273 cohort and 7 to 105-fold in the BNT162b2 cohort. The booster dose elicited a 2 to 238-fold increase in the mRNA-1273 cohort and a 20 to 255-fold increase in the BNT162b2 cohort from the lowest titer. These results replicate the antibody waning and recall trends following second and third doses reported in larger studies using serum specimens, supporting the use of non-invasive saliva specimens to monitor antibody titers during future epidemics or vaccination campaigns.

Introduction

The high efficacy of the BNT162b2 (Pfizer-BioNTech)[1]and mRNA-1273 (Moderna)[2] lipid-nanoparticle encapsulated messenger RNA (mRNA) vaccines at preventing COVID-19 infection, severe disease, hospitalizations, and death, and the subsequent rapid global vaccination campaign, offered hope that herd-immunity to SARS-CoV-2 could be attained, and the COVID-19 pandemic successfully controlled. However, a combination of waning natural or vaccine-induced humoral immunity[3] and the evolution of SARS-CoV-2 variants led to decreased vaccine efficacy and increased breakthrough infections in fully vaccinated populations and reinfections in recovered populations[4]. Consequently, third-dose booster immunizations were assessed and implemented during a high incidence of infections globally to respond to the resurgent epidemic wave[5].

High immunoglobulin G (IgG) and protective neutralizing antibodies (nAb) in human serum are induced by natural infection or vaccination, but antibody (Ab) titers wane in the six months following the second dose of mRNA vaccines allowing increased SARS-CoV-2 infection rates in fully vaccinated populations[3]. The mRNA vaccines were designed against the ancestral SARS-CoV-2 Wuhan/HU-1/2019 strain prefusion stabilized full-length spike (S-) protein[6]. Consequently, antigenic evolution of the S-protein through point mutations and recurrent deletions particularly in the receptor binding domain (RBD) enabled immune evasion caused

by reduced Ab neutralization of new variants[7]. The emergence of immune-escape variants including the Omicron (B.1.1.529) BA.1 and BA.2 variants ensured herd immunity was not attainable and rendered monoclonal antibody therapies ineffective[8]. A third-dose homologous or heterologous booster vaccination with either mRNA-1272 or BNT162b2 mRNA vaccines reduced symptomatic COVID-19 infections, hospitalization, and death for Delta (B.1.617.2) and Omicron variants, although efficacy against asymptomatic and mild symptomatic Omicron variant infection was reduced[9,10]. The titer and breadth of the nAb response against the ancestral strain and both variants were significantly recalled in individuals following a homologous third-dose booster vaccination, although titers were lower against the variants, indicating cross-reactive broadly neutralizing nAbs constitute a subset of all nAbs[11,12]. Nevertheless, breakthrough infections with the Omicron variant following booster vaccinations were reported for individuals within weeks of a third dose[13].

Understanding Ab responses to natural SARS-CoV-2 infection or vaccination by enzyme-linked immunosorbent assay (ELISA) assays has primarily relied on patient serum derived from blood, but this has limitations associated with the invasiveness of the procedure and specimen processing[14]. Alternatively, oral mucosal fluid (saliva) specimens are non-invasive, enable self-collection, and require less processing, reducing cost[14]. Multiple studies demonstrated that anti-S-protein IgG levels can be robustly measured in saliva specimens with high sensitivity and specificity from infected or vaccinated individuals, even though salivary Ab concentrations are 100-10,000-fold lower than in serum[15,16]. IgG titers for serum and saliva are strongly correlated, indicating they represent a surrogate for anti-S-protein IgG levels in serum[15,16]. In contrast, anti-S-protein IgA Abs are not reliably detected in saliva, although serum IgA titers increase in response to infection or vaccination and are consistently detected in specimens[16].

We previously reported the anti-S-protein IgG Ab response in two cohorts who received homologous vaccination regimens with two doses of either the mRNA-1273 mRNA or BNT162b2 mRNA vaccine, in which participants performed self-collection of saliva using the OraSure®oral fluid collection device at pre-determined time-points for ninety days[17]. We report new results from those participants who received a third-dose booster and continued to perform self-collection of saliva at pre-determined time-points for up to fifteen months following the first vaccination.

Material and Methods

In our original clinical study, enrolled participants in the mRNA-1273 and BNT162b cohorts provided one self-collected saliva specimen for anti-S-protein IgG testing following their first and second vaccination doses at predetermined time intervals and every thirty days thereafter[17]. Booster study enrollment was offered to participants who had been enrolled in our previous two-dose regimen study[17]. Recruitment took place among individuals who were due to receive the third-dose booster vaccine of either the mRNA-1273 or BNT162b2 vaccines, constituting two independent cohorts.

Participants enrolled in the booster study in the mRNA-1273 cohort provided a saliva specimen 46 days after vaccination, on average, and every 30 days thereafter for 7 months. Participants enrolled during their third vaccine dose of the BNT162b2 vaccine provided a specimen a few days before, or on the day of vaccination, as well as on days 5, 11, 16, 20, 30, 60, 90, and every 30 days thereafter. Participants who received a positive Ab test at the first time point, or prior to enrollment, or who missed one collection time point, were excluded from the original clinical study. During the booster study, participants who received a positive PCR test before or after receiving the booster vaccine were excluded from the study.

The anti-SARS-CoV-2 IgG ELISA was performed, as previously described[17]. The Limit of Detection (LOD) was 1 ng/mL, and the Limit of Quantification (LOQ) was 1.5 ng/mL. All statistical analysis, and plots were generated using GraphPad Prism Software (GraphPad Prism, San Diego, USA). Paired t-tests were used to compare the second and third vaccine dose peak anti-S-protein IgG titers for each cohort.

Results

In January 2021, 124 participants who had received the mRNA-1273 vaccine within the last 5 days were

enrolled in the original study. During the complete two-dose regimen study period the mRNA-1273 study cohort was comprised of 39 participants, as 61 participants were excluded from the study due to incomplete sample return and 24 were excluded due to receiving a positive COVID-19 diagnosis during the study. Subsequently, 28 participants received the homologous third-dose mRNA-1273 booster vaccination, who had an average age of 29 years, including 23 (74.1%) participants who self-identified as female as assigned at birth.

In March 2021, 123 participants were enrolled in the original study on the day they received the BNT162b2 vaccine. During the complete two-dose regimen study period the BNT162b2 study cohort was comprised of 41 participants, as 67 participants were excluded from the study due to incomplete sample return and 15 were excluded due to receiving a positive COVID-19 diagnosis during the study. Subsequently, 29 participants received the homologous third-dose BNT162b2 booster vaccination cohort, who had an average age of 50 years, including 17 (58.6 %) participants who self-identified as female as assigned at birth.

Participants in our longitudinal study received their homologous booster vaccination as it became widely available, 7-10 months after their second dose. We report the results for only the participants who received the homologous third-dose booster vaccination and reached the booster study endpoint. The second dose peak anti-S-protein IgG titer was 750 ± 362 ng/ml for mRNA-1273 and 591 ± 842 ng/mL for BNT162b2, so highly variable between individuals. Anti-S-protein IgG titers gradually waned from the peak after the second dose over the subsequent months in individuals by 9-150-fold for mRNA-1273 (Fig. 1), and 7-105-fold for BNT162b2 (Fig. 2) vaccines. However, anti-S-protein IgG was still robustly detected for all participants above the limit of quantification of the ELISA assay for mRNA-1273 (23 ± 16 ng/mL) and BNT162b2 (12 ± 9 ng/mL). These results reflect the waning trend reported for serum anti-S-protein IgG Ab, as well as variability in humoral responses in the study participants.

The booster vaccine increased the concentrations of anti-S-protein IgG levels in the saliva of all study participants in both cohorts. Soon after receiving the third-dose booster, peak anti-S-protein IgG levels for both cohorts attained that of the initial two-dose vaccination regime, showing robust recall of these salivary Abs (Fig. 1 and 2). The mean third dose peak anti-S-protein IgG titers was 801 ± 556 ng/mL for mRNA-1273 (Fig. 3) and 1377 ± 1633 ng/mL for BNT162b2 (Fig. 4). The timing of the third-dose booster varied between individuals, thus increasing the spread of the dataset. The mean peak anti-S-protein IgG titers between second and third dose were statistically significant ($p = 0.0021$) for the BNT162b2 cohort (Fig. 4), but not the mRNA-1273 cohort ($p = 0.666$) (Fig. 3). We followed these participants for 8 months after booster vaccination. Anti-S-protein IgG titers waned after the third dose peak for both cohorts following a similar trend to that of the initial two-dose regimen (Fig. 1 and 2). However, we robustly detected anti-S-protein IgG levels above the limit of quantification of the ELISA assay with a mean of 217 ± 288 ng/mL for mRNA-1273 (Fig. 1) after 211 days and a mean of 108 ± 127 ng/mL for BNT162b2 after 235 days (Fig. 2). These results show the rapid recall of the peak IgG titer in saliva following the third-dose booster, before waning again, offering a sustained level of Ab protection for participants.

Discussion

We report the combined results from two longitudinal prospective clinical studies measuring SARS-CoV-2 anti-S-protein IgG titers by ELISA in self-collected saliva following homologous two-dose vaccine regimen and subsequent third-dose booster vaccination with either mRNA-1273 and BNT162b2. We only included those participants in the analysis who received the homologous third-dose booster and completed the entire self-collection series. Our results for BNT162b2 and mRNA-1273 vaccine show IgG titers in saliva wane gradually from the second dose peak in the subsequent 6-9 months up to 150-fold. These results correlate with efficacy studies demonstrating that protection against a new SARS-CoV-2 infection and serum IgG titers wane over time in uninfected recipients of two doses of mRNA vaccines[4]. Administration of a third dose stimulates a robust recall of anti-S-protein IgG titer before waning in the subsequent months.

The approval and rollout of mRNA-1273 and BNT162b2 vaccine booster doses in September 2021 preceded the emergence of the omicron wave of infections during December 2021 in the USA, and fully vaccinated

individuals with a booster dose had a lower COVID-19 case rate than recipients of the two-dose regimen, or unvaccinated individuals[18]. However, vaccine effectiveness of the booster dose against symptomatic infection by omicron was reduced compared to the primary series against earlier variants, and protection further declined within five to nine weeks of vaccination[18,19]. Therefore, break-through infections of participants in our clinical study led to increased drop-out rate over its duration. Twenty-four mRNA-1273 vaccine recipients and fifteen BNT162b2 vaccine recipients were excluded from our study due to COVID-19 infection, of these, sixteen (66.7 %) and nine (60 %) participants self-reported a COVID-19 infection after receiving a booster dose of their respective vaccines. Four study participants (two mRNA-1273 and two BNT162b2 vaccine recipients) had an unexplained increase in anti-S-protein IgG titers, months after a booster vaccination, since none self-reported a COVID-19 infection, we suspect these to be asymptomatic infections. In addition, a high percentage of participants were unenrolled over time due to missed sample collection (49 % mRNA-1273 and 54 % BNT162b2 cohorts). This could be attributed to our clinical study requiring multiple collections over varying amounts of time, whereas a consistent collection cycle could have encouraged more participants to remain enrolled.

Finally, the updated bivalent fourth dose (second booster) became available September 2nd, 2022, in the USA, but our study reached its endpoint in May 2022. Both bivalent booster mRNA vaccines encode the S-protein antigen for the original Wuhan strain as well as for the BA.4 and BA.5 omicron variants for BNT162b2, and the BA.1 omicron variant for mRNA-1273.214[20]. Therefore, these updated bivalent booster mRNA vaccines induce a greater diversity of anti-S-protein Abs in recipients. We would expect saliva specimens to continue to be useful for measuring anti-S-protein IgG titers for this bivalent booster, and every future booster, but the ELISA assay would need updating with a mutated S-protein antigen to retain its high sensitivity.

Limitations

Collection of matched serum and saliva specimens from each participant for the entire duration of the combined mRNA vaccine studies would have provided more persuasive evidence supporting the correlation of serum and saliva anti-S-protein IgG titers over time. However, this was not practical, and would have decreased participation. Nevertheless, we previously demonstrated our ELISA assay using saliva specimens had comparable sensitivity and specificity to serum-based assays[15].

Also, the continuous evolution of the S-protein in omicron and other new variants could have reduced the sensitivity of the ELISA assay to detect heterogeneous anti-S-protein IgG increases from natural infections.

Conclusions

Our results demonstrate the value of the self-collection of saliva specimens using the OraSure® oral fluid collection device in longitudinal clinical studies for viral IgG titer measurement by ELISA during an epidemic or vaccination campaign.

Author Contributions

Robert W.B. Brown: writing – original draft preparation (lead); **Prithivi Chellamuthu:** conceptualization (supporting), methodology, writing – review and editing; **Edwyn Saavedra:**methodology, investigation (equal), data curation (equal), formal analysis (equal), visualization (lead); **Nicholas Denny:**methodology, investigation (equal), data curation (equal), formal analysis (equal); **Marilisa Santacruz:** investigation (equal), data curation (supporting); **Aaron N. Angel:** investigation (equal), data curation (supporting); **Melanie A. MacMullan** : project administration (lead); **Maria Shacreaw** : resources (supporting); **Lauren Lopez** : resources (supporting);**Victoria Hess** : resources (supporting); **Noah Kojima:**conceptualization (supporting); **Jeffery D. Klausner:**conceptualization (supporting); **Matthew Brobeck:** resources (supporting); **Frederick Turner:** funding acquisition (equal), resources (lead); **Vladimir I. Slepnev:** funding acquisition (equal); conceptualization (lead); **Albina Ibrayeva:**conceptualization (supporting), resources (supporting); supervision (lead).

Data Availability Statement

Not applicable.

Conflicts of Interest

Noah Kojima served as independent consultants for Curative Inc. All authors are or were at the time of the research, employed by Curative Inc. Jeffrey D. Klausner served as an independent medical director of Curative Inc. F.T, V.S, and R.W.B.B. are shareholders of Curative Inc.

Ethics Statement

The Advarra Institutional Review Board approved this clinical study (IRB# Pro00048737). The Advarra IRB waived the requirement for signed informed consent for the research under 45 CFR 46.117(c)(2). The study team complied with all Advarra IRB policies and procedures, as well as with all applicable Federal, State, and local laws regarding the protection of human subjects in research as stated in the approved IRB (Pro00048737).

Funding

Curative Inc. funded this research

Correspondence

ORCID

Robert W.B. Brown https://orcid.org/0000-0003-4086-7699

Edwyn Saavedra https://orcid.org/0000-0003-4583-4001

Melanie MacMullan https://orcid.org/0000-0001-5296-5902

Lauren Lopez https://orcid.org/0000-0002-4574-3524

Noah Kojima https://orcid.org/0000-0002-3667-9719

Jeffrey D. Klausner https://orcid.org/0000-0002-6922-7364

Fred E. Turner https://orcid.org/0000-0003-3307-9651

Albina Ibrayeva https://orcid.org/0000-0001-5782-5511

References

1. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **383** , 2603–2615 (2020).

2. Baden, L. R. *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N. Engl. J. Med.* **384** , 403–416 (2021).

3. Levin, E. G. *et al.* Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. *N. Engl. J. Med.* **385** , e84 (2021).

4. Goldberg, Y. *et al.* Protection and Waning of Natural and Hybrid Immunity to SARS-CoV-2. *N. Engl. J. Med.* **386** , 2201–2212 (2022).

5. Bar-On, Y. M. *et al.* Protection against Covid-19 by BNT162b2 Booster across Age Groups. *N. Engl. J. Med.* **385** , 2421–2430 (2021).

6. Corbett, K. S. *et al.* SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* **586** , 567–571 (2020).

7. Harvey, W. T. *et al.* SARS-CoV-2 variants, spike mutations and immune escape. *Nat. Rev. Microbiol.* **19** , 409–424 (2021).

8. Uraki, R. *et al.* Characterization and antiviral susceptibility of SARS-CoV-2 Omicron/BA.2. *Nature* (2022) doi:10.1038/s41586-022-04856-1.

9. Abu-Raddad, L. J. *et al.* Effect of mRNA Vaccine Boosters against SARS-CoV-2 Omicron Infection in Qatar. *N. Engl. J. Med.***386** , 1804–1816 (2022).

10. Barda, N. *et al.* Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. *Lancet Lond. Engl.* **398** , 2093–2100 (2021).

11. Garcia-Beltran, W. F. *et al.* mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell* **185** , 457-466.e4 (2022).

12. Chu, L. *et al.* Immune response to SARS-CoV-2 after a booster of mRNA-1273: an open-label phase 2 trial. *Nat. Med.* **28** , 1042–1049 (2022).

13. Kuhlmann, C. *et al.* Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet Lond. Engl.***399** , 625–626 (2022).

14. Casian, J. G. *et al.* Saliva-Based ELISAs for Effective SARS-CoV-2 Antibody Monitoring in Vaccinated Individuals. *Front. Immunol.* **12** , 701411 (2021).

15. MacMullan, M. A. *et al.* Detection of SARS-CoV-2 Antibodies in Oral Fluid Obtained Using a Rapid Collection Device. *J. Clin. Microbiol.* **59** , e02510-20 (2021).

16. Thomas, S. N. *et al.* Ultrasensitive detection of salivary SARS-CoV-2 IgG antibodies in individuals with natural and COVID-19 vaccine-induced immunity. *Sci. Rep.* **12** , 8890 (2022).

17. Mades, A. *et al.* Detection of persistent SARS-CoV-2 IgG antibodies in oral mucosal fluid and upper respiratory tract specimens following COVID-19 mRNA vaccination. *Sci. Rep.* **11** , 24448 (2021).

18. Johnson, A. G. *et al.* COVID-19 Incidence and Death Rates Among Unvaccinated and Fully Vaccinated Adults with and Without Booster Doses During Periods of Delta and Omicron Variant Emergence - 25 U.S. Jurisdictions, April 4-December 25, 2021. *MMWR Morb. Mortal. Wkly. Rep.* **71** , 132–138 (2022).

19. Andrews, N. *et al.* Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. *N. Engl. J. Med.* **386** , 1532–1546 (2022).

20. Chalkias, S. *et al.* A Bivalent Omicron-Containing Booster Vaccine against Covid-19. *N. Engl. J. Med.* **387** , 1279–1291 (2022).

FIGURES

Figure 1. Longitudinal time course of anti-S-protein IgG titers in saliva from uninfected participants who received three homologous doses of the mRNA-1273 (Moderna) vaccine (n = 28). Anti-S-protein IgG titers waned 9-150-fold in the months following the second dose of mRNA-1273. However, a robust recall 2 to 238-fold of anti-S-protein IgG titers in saliva occurred immediately following a third dose. The dotted line box denotes the timing participants received their third dose.

Figure 2. Longitudinal time course of anti-S-protein IgG titers in saliva from uninfected participants who received three homologous doses of the BNT162b2 (Pfizer-BioNTech) vaccine (n = 29). Anti-S-protein IgG titers waned 7-105-fold in the months following the second dose of BNT162b2. However, a robust recall 20 to 255-fold of anti-S-protein IgG titers in saliva occurred immediately following a third dose. The dotted line box denotes the timing participants received their third dose.

Figure 3. Anti-S-protein IgG titer peaks for second and third dose in mRNA-1273 cohort. The second versus third dose peaks were 750 ± 362 ng/mL and 801 ± 556 ng/mL (n = 28) which was not a statistically significant difference (p = 0.666).

Figure 4. Anti-S-protein IgG titer peaks for second and third dose in BNT162b2 cohort. The second versus third dose peaks were 591 ± 842 ng/mL and 1377 ± 1633 ng/mL (n = 29) which was a statistically significant difference (p = 0.0021).