

Neutralizing Antibody Activity to Severe Acute Respiratory Syndrome Coronavirus 2 Delta (B.1.617.2) and Omicron (B.1.1.529) After 1 or 2 Doses of BNT162b2 Vaccine in Infection-Naive and Previously Infected Individuals

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Previous reports demonstrated that severe acute respiratory syndrome coronavirus (SARS-CoV-2) binding immunoglobulin G levels did not increase significantly between the first and second doses of the BNT162b2 vaccine in previously infected individuals. We tested neutralizing antibodies (nAbs) against SARS-CoV-2 Delta and Omicron variants after the first and second doses of this vaccine in infection-naive and previously infected individuals. Delta, but not Omicron, nAb titers significantly increased from the first to the second dose in both groups of individuals. Importantly, we found that Omicron nAb titers were much lower than Delta nAb titers and that even after 2 doses of vaccine, 17 of 29 individuals in the infection-naive group and 2 of 27 in the previously infected group did not have detectable Omicron nAb titers. Infection history alone did not adequately predict whether a second dose resulted in adequate nAb. For future variants of concern, the discussion on the optimal number of vaccine doses should be based on studies testing for nAb against the specific variant.

Keywords. SARS-CoV-2; vaccine; Delta; Omicron; variants of concern; neutralization; antibodies.

The SARS-CoV-2 variant B.1.617.2 (Delta) was first reported in the United States in May 2021. By August 2021, Delta

comprised almost 95% of all sequenced cases in the United States until the appearance of variant B.1.1.529 (Omicron) in December 2021, and then Omicron quickly became the dominant variant. There is a 3–5-fold decrease in neutralizing antibody (nAb) titers against Delta compared with B.1.1.7 (Alpha) in fully vaccinated, infection-naive individuals [1]. Delta nAb titers also are lower than D614G nAb titers in both infection-naive and previously infected individuals 4–6 weeks after the second dose of BNT162b2 vaccine [2]. nAb activity against Omicron is 20–30-fold lower than against ancestral SARS-CoV-2 after primary vaccination and is 3–5-fold lower after a boost [3, 4].

We and others have reported that SARS-CoV-2 anti-receptor-binding domain (RBD) or anti-spike immunoglobulin (Ig) G antibody levels did not increase significantly between the first and second doses of BNT162b2 in previously infected individuals [5–7]. These reports contributed to discussions as to whether a single vaccine dose might be sufficient in previously infected individuals [8] and may guide coronavirus disease 2019 (COVID-19) vaccination policies to maximize vaccine coverage and vaccine equity. Those previous studies [5–7] tested only for binding antibodies against wild-type (WT) virus. Given that nAb titers against Delta and Omicron are significantly lower than against the WT virus, a second dose of vaccine might be necessary even for previously infected individuals.

We tested available stored plasma samples from individuals who participated in our group's previously published study [7] to assess whether 2 doses of BNT162b2 induce higher Delta and Omicron nAb titers than 1 dose in previously infected and in infection-naive individuals. We also compared Delta and Omicron nAb responses to D614G nAb responses after 1 and 2 doses of vaccine.

METHODS

Regulatory Approval and Study Subjects

This study was approved by the Rush University institutional review board. All participants provided written informed consent. All individuals received their first dose of BNT162b2 between December 2020 and January 2021. Available plasma samples from 29 infection-naive and 27 previously infected individuals who participated in our group's previously published study [7] were tested for Delta, Omicron, and D614G nAbs. The samples were collected at 3 time points: 0–3 days before vaccination (baseline), 3 weeks after the first dose (and before to the second dose) and 4 weeks after the second dose of BNT162b2.

None of the 29 individuals in the infection-naive group had histories of COVID-19 symptoms, positive polymerase chain

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reaction (PCR) test results, or anti-RBD IgG at baseline. Among the 27 previously infected individuals, 22 had histories of COVID-19 symptoms and positive PCR results. Among these 22, 18 had positive anti-nucleocapsid and anti-RBD IgG at baseline, and 4 were negative for anti-nucleocapsid and anti-RBD IgG at baseline. Five individuals had positive anti-nucleocapsid IgG detected when they participated in a SARS-CoV-2 antibody screening research study in May 2020 (but never had COVID-19 symptoms or PCR testing). The 22 individuals with positive PCR results were infected between March and November 2020, when D614G was the dominant variant.

All 29 individuals in the infection-naive group had plasma samples available for testing at all 3 time points. In the previously infected group all 27 individuals had post-second-dose plasma samples tested, 24 had available plasma samples for testing at baseline, and 25 had available plasma samples after the first dose.

SARS-CoV-2 Pseudovirus Neutralization Assay

nAb titers were measured as a function of reductions in luciferase reporter gene expression after a single round of infection with SARS-CoV-2 D614G (or Delta or Omicron) spike pseudotyped virus in 293 T/angiotensin-converting enzyme 2 cells, as described elsewhere [9, 10]. Median infective dose (ID₅₀) nAb titers were calculated based on a dose-response curve. The limit of detection was an ID₅₀ of 20.

Anti-RBD IgG Assay

Anti-RBD (D614G) IgG levels were measured on an Abbott ARCHITECT i2000SR as described in our group's previously published study [7]. Results are reported in arbitrary units (AU) per milliliter, with values ≥ 50 AU/mL considered positive.

Statistical Analysis

Statistical analysis was conducted with Prism software, version 8.02, using the 2-tailed nonparametric Mann-Whitney test for comparison between 2 groups and Spearman rank correlation to assess correlation between SARS-CoV-2 IgG levels and nAb titers (ID₅₀).

RESULTS

In the infection-naive group, 14 (48.3%) individuals were white and 22 (75.9%) were female, with a mean age of 42.7 years (standard deviation, 11.6 years; range, 28–65 years); in the previously infected group, 18 (66.7%) individuals were white, and 19 (70.4%) were female, with a mean age of 42.4 years (11.4 years; 22–64 years). [Figure 1A](#) shows that after 2 vaccine doses, Delta and Omicron nAb titers were significantly lower than D614G nAb titers in both groups. The geometric means (95% confidence interval) for Delta, Omicron, and D614G

nAb ID₅₀ values were 275.4 (201–377.4), 17.14 (13.0–22.6), and 602.6 (499.7–726.7), respectively (2.19-fold decrease for Delta and 35.16-fold decrease for Omicron; both $P < .001$) in the infection-naive group and 915 (578.8–1447), 352 (177–699), and 2526 (1727–3693), respectively (2.76-fold decrease for Delta and 7.18-fold decrease for Omicron; both $P < .001$) in the previously infected group.

The left panel of [Figure 1A](#) shows that post-second-dose Delta nAb titers were heterogeneous in the infection-naive group (coefficient of variation, 83%; ID₅₀ range, 50.1–1323.8) and more heterogeneous ([Figure 1A, right panel](#)) in the previously infected group (coefficient of variation, 284%, ID₅₀ range, 120.3–37936.3). Omicron nAb titers were similarly heterogeneous. For post-second-dose Omicron nAb titers, the coefficient of variation and ID₅₀ range were 92.67 and 10–88, respectively, in the infection-naive group and 155.4% and 10–7942 in the previously infected group.

[Figure 1B](#) shows that D614G nAb titers increased significantly (+12.18-fold; $P < .001$) from after the first to after the second dose in the infection-naive group. However, there was no significant increase (+1.88-fold; $P = .57$) in the previously infected group. Delta nAb titers increased from after the first to after the second dose in both the infection naive (+7.57-fold; $P < .001$) and the previously infected (+2.48-fold; $P = .04$). Omicron nAb titers did not increase from after the first dose to after the second-dose in either the infection-naive (+1.48-fold; $P = .11$) or the previously infected groups (+1.05-fold; $P = .96$). After the first vaccine dose, 10 individuals in the infection-naive and 2 in the previously infected group did not have detectable Delta nAb titers, and 25 in the infection-naive and 3 in the previously infected group did not have detectable Omicron nAb titers. After the second dose, all individuals in both groups achieved detectable Delta nAb titers. However, even after 2 doses of vaccine, 17 individuals in the infection-naive group and 2 in the previously infected group did not have detectable Omicron nAb titers.

There were no significant differences between men and women in anti-RBD IgG levels or D614G, Delta, and Omicron nAb in either the infection-naive or the previously infected group. Anti-RBD IgG data generated from our group's previously published report [7] were used to determine correlations between age and anti-RBD IgG levels. In the infection-naive group, age was negatively correlated with anti-RBD IgG levels after both doses of vaccine ($r = -0.65$ and $P < .001$ after the first dose; $r = -0.46$ and $P = .01$ after the second dose) and with D614G nAb titers ($r = -0.53$ and $P = .003$ after the first dose; $r = -0.41$ and $P = .03$ after the second dose) (data not shown). There was no correlation between age and Delta or Omicron nAb titers after either dose in the infection-naive group.

[Figure 1C](#) shows that in the previously infected group, all correlation values were positive for age and nAb titers for D614G, Delta, and Omicron. However, after the second vaccine

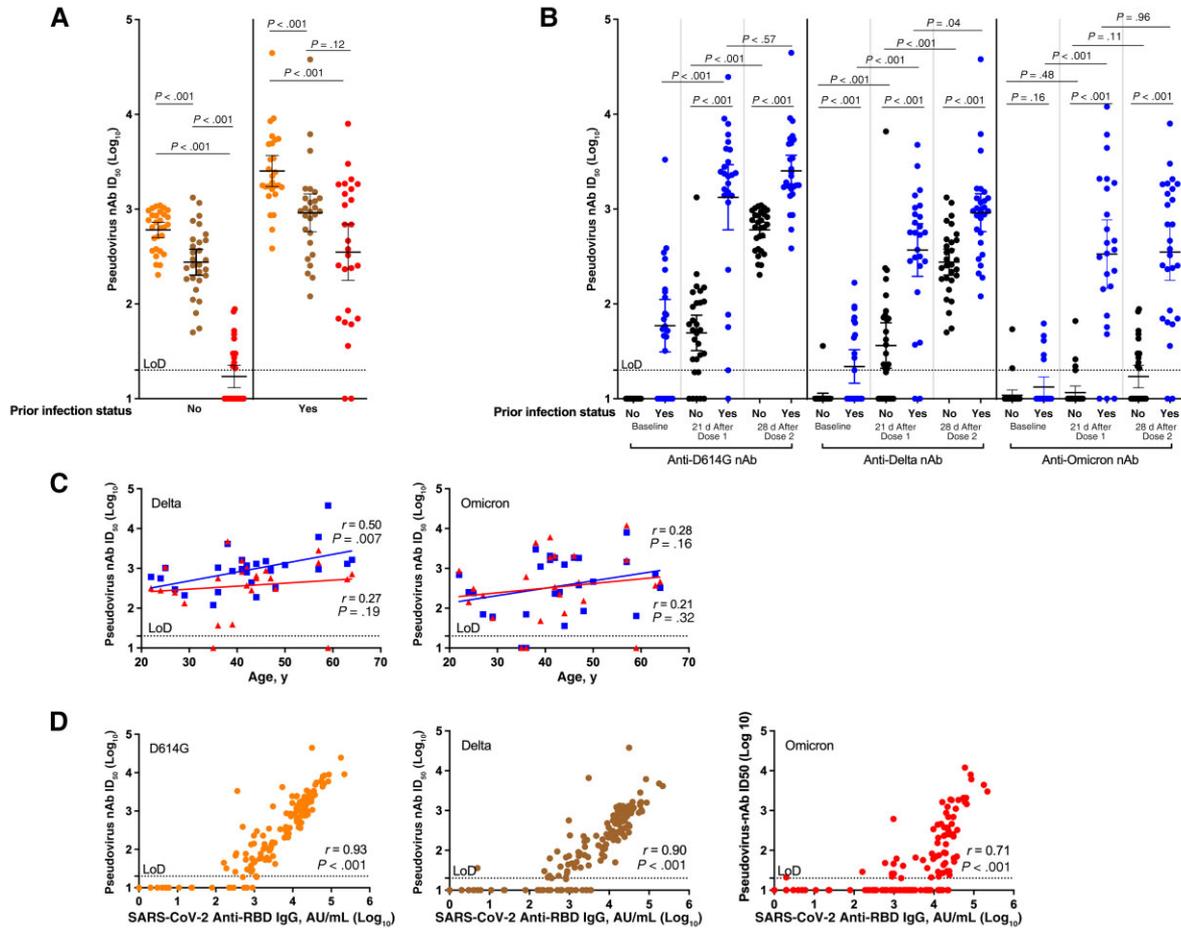


Figure 1. Plasma neutralizing antibody (nAb) titers (expressed as median infective dose [ID₅₀]) against D614G, Delta, and Omicron severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, in infection-naive and previously infected individuals vaccinated with BNT162b2. *A*, nAb titers against D614G (orange dots), against Delta (brown dots), and against Omicron (red dots) 28 days (4 weeks) after the second dose of BNT162b2 vaccine in infection-naive ($n = 29$; left panel) and previously infected ($n = 27$; right panel) individuals. Dashed line represents the limit of detection (LoD; ID₅₀, 20); solid bars, geometric means; and error bars, 95% confidence intervals. Statistical analysis was performed using the 2-tailed nonparametric Mann-Whitney test to compare the 2 groups. *B*, nAb titers to D614G (left panel), Delta (middle panel), and Omicron (right panel) before (baseline) and after 1 or 2 doses of BNT162b2 vaccine in infection-naive (black dots) and previously infected (blue dots) individuals. Statistical analysis was performed using the 2-tailed nonparametric Mann-Whitney test to compare 2 time points. *C*, Correlation of age with D614G, Delta and Omicron nAb in previously infected individuals after 1 dose (red triangles) or 2 doses (blue boxes) of BNT162b2 vaccine. Correlation was assessed using Spearman rank correlation. *D*, Correlation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-receptor-binding domain (RBD) immunoglobulin (Ig) G binding levels (in arbitrary units [AU] per milliliter) with D614G (orange dots), Delta (brown dots), and Omicron (red dots) nAb titers (ID₅₀). All RBD IgG and nAb data ($n = 163$) from baseline and after the first and second vaccine doses were used to assess correlation between SARS-CoV-2 IgG and nAb titers, using Spearman rank correlation.

dose, the only significant correlation was for Delta ($r = 0.50$; $P = .007$) (Figure 1C, blue boxes).

Analysis of all data for all subjects at all time points showed that anti-RBD IgG levels were correlated well with nAb titers for D614G ($r = 0.931$), Delta ($r = 0.90$) and Omicron ($r = 0.71$) (all $P < .001$) (Figure 1D). Delta nAb and Omicron nAb titers were also correlated well with D614G nAb titers ($r = 0.91$ and $r = 0.76$, respectively; both $P < .001$; data not shown).

DISCUSSION

These data show that Delta nAb titers, and to a greater extent, Omicron nAb titers are lower than D614G nAb titers in both

infection-naive and previously infected individuals after 2 doses of BNT162b2. Others have reported similar decreases in Delta and Omicron nAb titers compared with D614G or Alpha nAb titers after 2 or 3 doses of BNT162b2 [1–4]. Achieving high vaccination rates worldwide is the best strategy against the SARS-CoV-2 pandemic. In many countries the availability of vaccines is low. To make the best possible use of the scarce vaccine supply, some investigators [5, 8] have questioned whether a single vaccine dose might be sufficient to induce adequate antibody titers in individuals with a history of COVID-19. Our results show that even for previously infected individuals a second dose increases Delta nAb titers above a single dose. Thus, it cannot be predicted whether 1 dose is

adequate to achieve maximal nAb titers against future variants in previously infected individuals.

In addition, our data showed that the Delta and Omicron nAb responses to vaccination were heterogeneous in both the infection-naïve and previously infected groups, and it cannot be assumed that all previously infected individuals would have high Delta or Omicron nAb titers. Two previously infected individuals in the current study had undetectable Delta nAb titers after the first dose and needed a second dose to achieve detectable titers. More strikingly, after 2 doses of vaccine, 17 individuals in the infection-naïve group and 2 in the previously infected group did not have detectable Omicron nAb titers. These 19 individuals need to be evaluated to determine if a third vaccine dose would result in detectable Omicron nAb titers.

For the individuals in the current study, infection history did not adequately predict whether an individual required a second dose to achieve detectable or adequate nAb titers against Delta or Omicron. Our results underscore the fact that infection- and vaccine-induced nAb activities against the different SARS-CoV-2 variants vary greatly. Given that the Omicron variant has replaced the Delta variant and new variants are on the rise, nAb studies with the latest variants are needed to determine whether previous infection status has any bearing on vaccination strategies.

Age has been shown to be negatively correlated with SARS-CoV-2 spike IgG, anti-RBD IgG, and WT and P.1 variant nAbs after BNT162b2 vaccination [11–14]. In the current study, we also found that anti-RBD IgG and D614G nAbs were negatively correlated with age in the infection-naïve group. However, there was no correlation with age and anti-RBD IgG or D614G nAbs in the previously infected group. Surprisingly, we found that age was positively correlated with Delta (but not Omicron) nAbs after the second vaccine dose in the previously infected group. These divergent findings with respect to age and vaccine-induced antibody responses could reflect differences in the immune responses between infection-naïve and previously infected individuals and differences in antibody responses to different variants of SARS-CoV-2. Although positive correlation between age and anti-spike IgG levels has been reported with the second dose of the AZD1222 vaccine [14], our findings could be the result of a small sample size.

We did not find any sex differences in binding or nAb titers after vaccination. This is likely due to our study's small sample size and the fact that the majority (75%) of participants in our study were women.

Our data demonstrated that an anti-RBD IgG test with Food and Drug Administration emergency use authorization had good correlation with Delta and Omicron nAb titers. In large vaccine clinical trials, high levels of SARS-CoV-2 IgG binding antibodies and high nAb titers have been correlated with a reduced risk of symptomatic infection [9,15]. Because nAb assays

are mostly research assays, SARS-CoV-2 spike or RBD IgG tests performed in clinical laboratories might serve as readily available surrogate tests for nAbs. These assays will help determine whether an individual requires additional doses of vaccine, with the caveat that the clinical antibody tests have been correlated with nAb titers and with protection from serious SARS-CoV-2 disease.

Limitations of the study include the small sample size and low diversity of participants (with regard to age, sex, and race). Because of the small sample size, our finding that after the second dose, age was positively correlated with Delta nAb titers in the previously infected group might be due to chance or unmeasured confounding. However, it is interesting that all the r values for previously infected individuals (Figure 1C) were positive while all the r values were negative for correlation between age and antibody levels in the infection-naïve individuals. (data not shown) Because we did not evaluate vaccines other than BNT162b2, our findings should not be extended to other vaccines.

Notes

Author contributions. J. N. M. and A. L. conceived and designed the study. X. S. and D. C. M. performed the neutralization assays. M. S. performed the RBD immunoglobulin G Assays. J. N. M., M. A., X. S., J. F., A. G., D. N., G. C., and J. K. analyzed the data. J. N. M. and A.L. wrote the manuscript with input from all authors.

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Potential conflicts of interest. M. A., M. S., and G. C. are employees of Abbott Diagnostics. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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