Severe Acute Respiratory Syndrome Coronavirus 2 Neutralization After Messenger RNA Vaccination and Variant Breakthrough Infection

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The emergence of severe acute respiratory syndrome coronavirus 2 variants that have greater transmissibility and resistance to neutralizing antibodies has increased the incidence of breakthrough infections. We show that breakthrough infection increases neutralizing antibody titers to varying degrees depending on the nature of the breakthrough variant and the number of vaccine doses previously administered. Omicron breakthrough infection resulted in neutralizing antibody titers that were the highest across all groups, particularly against Omicron.

Keywords. breakthrough infection; neutralization; Omicron; SARS-CoV-2; vaccination.

Vaccination and infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants are currently shaping immunity to SARS-CoV-2 in individuals and populations [1]. The exposure of individuals to SARS-CoV-2 antigens has become highly heterogeneous as breakthrough infections of vaccinated or previously infected individuals with variants that evade preexisting immunity have become common. In particular, Omicron (B.1.1.529) has a large number of amino acid changes compared to ancestral SARS-CoV-2 and is highly resistant to plasma neutralizing antibodies elicited by infection with prior variants and the 2-dose messenger RNA (mRNA) vaccination regimens. Nevertheless, vaccination after infection or administration of a third mRNA vaccine dose elicits high levels of antibodies that have broad activity and can neutralize Omicron to a degree [2–5]. To assess the impact of breakthrough infection neutralizing antibody potency and breadth, we compared neutralizing antibody titers against the ancestral and recently emergent SARS-CoV-2 variants in 54 individuals who had received 2 or 3 doses of mRNA vaccine and experienced breakthrough infection with the Delta (B.1.617) or Omicron SARS-CoV-2 variants.

METHODS

Participants were healthy adults who had been vaccinated with 2 or 3 doses of an mRNA vaccine and experienced breakthrough infections with Delta (n = 24; median age, 30 years [range, 21–63 years]; 67% male, 33% female) or Omicron (n = 30; median age, 33.5 years [range, 22–79 years]; 53% male, 47% female). Samples were obtained between 13 August 2021 and 28 January 2022 for an average of 27 days (range, 14–55 days) after the latest positive test for infection (for details, see Supplementary Table 1). The study was performed in compliance with all relevant ethical regulations, and the protocol (DRO-1006) for studies with human participants was approved by the institutional review board of The Rockefeller University. Uninfected vaccine recipient participants have been previously described [3] (Supplementary Table 1).

Blood Sample Processing and Storage

Heparinized plasma and serum samples were aliquoted and stored at −20 °C or below. Before experiments, aliquots of plasma samples were heat inactivated (56 °C for 1 hour) and then stored at 4 °C.

Pseudotyped Virus Neutralization Assays

293T cells were transfected with pNL4-3ΔEnv-nanoluc and pSARS-CoV-2-Δ19 plasmids [6]. Forty-eight hours later, particles were harvested, filtered, and stored at −80 °C. The amino acid deletions and/or substitutions corresponding to SARS-CoV-2 variants were incorporated into a spike expression plasmid using synthetic gene fragments (Integrated DNA Technologies (IDT)) or overlap extension polymerase chain reaction–mediated mutagenesis and Gibson assembly. Specifically, the variant-specific deletions and substitutions introduced into the B1 sequence were verified by sequencing and included B.1.617 (Delta): T19R/Δ156-8/L452R/Δ614G/Δ156-8/L452R/T95I/D614G/P681R/D950N; and B.1.1.529 (Omicron): A76V/Δ69-70/T95I/Δ211/L212/mus214EPE/G339D/Δ371L/Δ373P/Δ375F/K417N/Δ440K/G446S/Δ771N/T478K/E484A/Q493K/Δ69-70/T95I/Δ211/L212/mus214EPE/G339D/Δ371L/Δ373P/Δ375F/K417N/Δ440K/G446S/Δ771N/T478K/E484A/Q493K/Δ69-70/T95I/Δ211/L212/mus214EPE/G339D/Δ371L/Δ373P/Δ375F/K417N/Δ440K/G446S/Δ771N/T478K/E484A/Q493K/
G498R/N501Y/Y505H/T547K/D614G/H655K/N679K/P681H/N746K/D796Y/N856K/Q954H/N969K/L981F.

All spike proteins used in the pseudotype neutralization assays had a 19-amino acid C-terminal deletion and included the R683G substitution, which disrupts the furin cleavage site and increases particle infectivity without grossly affecting antibody sensitivity.

Five-fold serially diluted plasma samples from vaccinated individuals were incubated with SARS-CoV-2 pseudotyped virus for 1 hour at 37 °C. The mixture was subsequently added to an HT1080-based cell line engineered to express human angiotensin-converting enzyme 2 (HT1080.ACE2 cl14) [6]. The starting serum dilution on cells was 1:50. Nanoluc luciferase activity in lysates was measured 48 hours postinoculation using the Nano-Glo Luciferase Assay System (Promega) with a Glomax Navigator (Promega). Relative luminescence units were normalized to those derived from cells infected with SARS-CoV-2 pseudotyped virus in the absence of serum. The half-maximal neutralization titers for sera (NT_{50}) were determined using 4-parameter nonlinear regression (least squares regression method without weighting; constraints: top = 1, bottom = 0) (GraphPad Prism), and median values were calculated for each sample from 2–3 independent experiments. Group comparisons were performed using Mann-Whitney test (GraphPad Prism).

RESULTS

We collected samples from 54 individuals who had received 2 or 3 doses of mRNA-1273 (Moderna) or BNT162b2 (Pfizer) mRNA vaccines and were subsequently infected with SARS-CoV-2. Volunteer age was between 21 and 79 years (median age, 30 years); 32 (59%) were male and 22 (41%) were female (Supplementary Table 1). Breakthrough infection variants were deduced based on the prevalent variant circulating in New York City at the time of infection (Figure 1A; Supplementary Table 1). Specifically, Delta (B.1.617) was the predominant variant from July to early December 2021 but was rapidly displaced by Omicron by January 2022 (Figure 1A). As a consequence of evolving vaccination recommendations, 24 participants who experienced breakthrough infection with Delta had received 2 vaccine doses. Conversely, most Omicron breakthrough infections (24 of 30) were in participants who had received 3 vaccine doses.

In participants who did not experience breakthrough infection and received 2 vaccine doses, NT_{50} values against Delta and Omicron were 5.2- and 82.6-fold, respectively, lower than those against Wuhan-Hu-1, whereas in those who had received 3 vaccine doses, they were 9.2- and 16.9-fold lower (P < 0.0001 for all comparisons) (Figure 1B).

Participants who received 2 vaccine doses and had Delta breakthrough infection had median (range) NT_{50} values of 21 907 (7089–133 843), 9264 (2131–46 555), and 3000 (605–13 749) against Wuhan-Hu-1, Delta, and Omicron, respectively (Figure 1B). These values were 2.8-, 4.9-, and 26.4-fold greater than uninfected 2-dose vaccine recipients, for whom the corresponding NT_{50} values were 7628 (2299–50 640), 1453 (606–2666), and 92.3 (25–327) against Wuhan-Hu-1, Delta, and Omicron, respectively (all comparisons P ≤ .0005). Participants who received 3 vaccine doses and had Omicron breakthrough infection had median (range) NT_{50} values of 45 348 (21 661–300 044), 9724 (4829–73 494), and 11 380 (2197–109 591) against Wuhan-Hu-1, Delta, and Omicron, respectively (Figure 1B). By comparison, uninfected 3-dose vaccine recipients had median (range) NT_{50} values against these 3 viruses of 65 617 (15 641–341 247), 7079 (2978–18 768), and 3872 (1411–21 301). Therefore, Omicron breakthrough infection increased titers by 2.9-fold (P < 0.0001) against Omicron and 1.4-fold (P = 0.0088) against Delta. For the few individuals with Omicron breakthrough infection after receiving only 2 vaccine doses, median (range) NT_{50} values were 32 052 (8880–53 494), 10 749 (2333–16 327), and 14 911 (845–17 756) against Wuhan-Hu-1, Delta, and Omicron, respectively.

DISCUSSION

The repeated emergence of new SARS-CoV-2 variants such as Delta and Omicron, with increasing levels of resistance to neutralizing antibodies, has led to an increase in incidence of breakthrough infections in vaccinated individuals [7, 8]. Erosion of the efficacy of vaccines based on the ancestral Wuhan-Hu-1 strain may impact future vaccination strategies. We determined the effect of breakthrough infection on neutralizing antibodies against original and variant SARS-CoV-2. Study limitations include the relatively small number and heterogeneity of participants that reflect the real-world circumstances at the time of recruitment. We note that correlation between neutralizing antibody levels and protection has been reported [9], even though additional correlates remain to be defined.

Consistent with prior reports from our group and others [3–5, 10–12], we show that plasma neutralizing antibodies in recipients of 2 mRNA vaccine doses are less potent against Delta and particularly against Omicron. However, administration of a third mRNA vaccine dose significantly increases

2 • OFID • BRIEF REPORT
neutralizing antibody titers against both variants (Figure 1B). Our data further demonstrate that in individuals who received 2 mRNA vaccine doses, breakthrough infection by either Delta or Omicron increases neutralizing antibody titers against both variants. While the magnitude of the increase against Delta is comparable in individuals who experienced either Delta or Omicron breakthrough infection, the increase in neutralization potency against Omicron is 161.5-fold in Omicron breakthrough infections compared to 26.4-fold in Delta breakthrough infections. Compared to individuals who received 3 mRNA vaccine doses, Omicron breakthrough infections also generated increased neutralizing antibody titers, but the magnitude of the effect was small.

Because Omicron breakthrough infection in 2-dose vaccine recipients resulted in broader plasma neutralization activity than Delta breakthrough infection, our data suggest that an Omicron-specific booster shot may have similar effects in those who received only 2 mRNA vaccine doses [12]. In individuals who have already received a third dose of the currently available mRNA vaccines, Omicron-specific boosters may modestly improve neutralizing responses against Omicron while not significantly impacting neutralization of other variants.
Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. P. D. B. has received compensation from Pfizer for consultation in the area of SARS-CoV-2 vaccines. All other authors report no potential conflicts of interest.

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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