

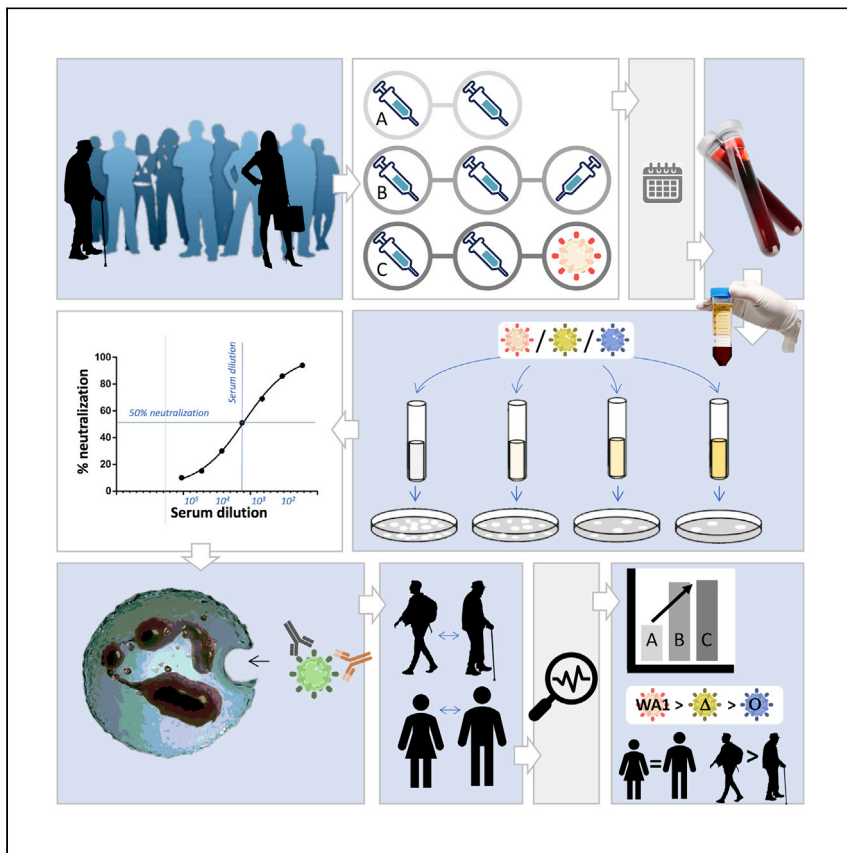


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Clinical and Translational Article

Omicron neutralizing antibody response following booster vaccination compared with breakthrough infection



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Highlights

SARS CoV-2 antibody responses rise after boosting and after breakthrough infection

Ab responses correlate negatively with age after vaccination but not after breakthrough

Ab-dependent cell-mediated phagocytosis rises 2-fold after boosting or breakthrough

Delta and Omicron BA.1 and BA.2 are progressively less susceptible to neutralization

The nature of immune responses following 2-dose and 3-dose vaccination and breakthrough infection remains to be fully investigated. Curlin et al. show that boosted vaccine regimens and breakthrough infection enhance immune responses similarly, but there is a progressive loss of efficacy against newly emerging variants.



Translation to Patients

Clinical and Translational Article

Omicron neutralizing antibody response following booster vaccination compared with breakthrough infection

Marcel E. Curlin,^{1,4,5,*} Timothy A. Bates,^{2,4} Gaelen Guzman,² Devin Schoen,¹ Savannah K. McBride,² Samuel D. Carpenter,³ and Fikadu G. Tafesse^{2,*}

SUMMARY

Background: The spread of the vaccine-resistant Omicron severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants threatens unvaccinated and fully vaccinated individuals, and accelerated booster vaccination campaigns are underway to mitigate the ongoing wave of Omicron cases. The immunity provided by standard vaccine regimens, boosted regimens, and immune responses elicited by vaccination plus natural infection remain incompletely understood. The magnitude, quality, and durability of serological responses, and the likelihood of protection against future SARS-CoV-2 variants following these modes of exposure, are poorly characterized but are critical to the future trajectory of the coronavirus disease 2019 (COVID-19) pandemic.

Methods: Ninety-nine individuals were semi-randomly selected from a larger vaccination cohort following vaccination and, in some cases, breakthrough infection. We analyzed spike receptor-binding domain-specific immunoglobulin G (IgG), IgA, and IgM by enzyme-linked immunosorbent assay, neutralizing antibody titers against live SARS-CoV-2 variants, and antibody-dependent cell-mediated phagocytosis.

Findings: In 99 vaccinated adults, compared with responses after two doses of an mRNA regimen, the immune responses 3 months after a third vaccine dose and 1 month after breakthrough infection due to prior variants show dramatic increases in magnitude, potency, and breadth, including increased antibody-dependent cellular phagocytosis and robust neutralization of the currently circulating Omicron BA.2 variant.

Conclusions: Boosters and natural infection substantially boost immune responses. As the number of Omicron sub-variant cases rise and as global vaccination and booster campaigns continue, an increasing proportion of the world's population will acquire potent immune responses that may be protective against future SARS-CoV-2 variants.

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INTRODUCTION

Since 2020, the global coronavirus disease 2019 (COVID-19) pandemic has been punctuated by episodic waves of increased incidence associated with the emergence of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with progressively greater transmissibility and resistance to immune responses elicited by currently approved vaccines. The most epidemiologically important

CONTEXT AND SIGNIFICANCE

Nearly 3 years since its appearance on the world stage, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic continues to accrue new cases despite an unprecedented global vaccine campaign. As more of the world population becomes exposed through vaccination and/or infection, it is hoped that rising population-level immunity will eventually quell the epidemic. However, there is currently an incomplete understanding of how immune responses resulting from standard and boosted vaccine regimens differ from responses resulting from breakthrough infection. Researchers at the Oregon Health and Sciences University show that 3-dose regimens and breakthrough infection enhance serological responses similarly, with higher neutralizing antibody titers and a greater ability to neutralize SARS-CoV-2 variants. Newly emerging variants display progressively less susceptibility to neutralization, highlighting both the continued relevance of current vaccines and the need for newly formulated vaccines directed at emerging SARS-CoV-2 variants.

variants have been classified as variants of concern (VOCs) by the World Health Organization and include Alpha, Beta, Gamma, Delta, and Omicron. The Omicron variant includes several competing sub-lineages including BA.1, BA.2, BA.4, BA.5, and BA.2.12.1, the latter four of which are presently responsible for most new cases. All Omicron sub-lineages are notable for their high transmissibility and resistance to neutralization by vaccine-induced antibodies.^{1–3} Each contain more than 60 amino acid changes relative to the founding strain, with more than 30 in the spike protein, and 15–17 falling within the receptor-binding domain (RBD) responsible for binding to the human cell surface receptor angiotensin-converting enzyme 2 (ACE2).⁴ All known neutralizing antibodies bind to the spike protein, with the vast majority targeting the RBD.^{5–7} Mutations within this region have caused a dramatic decrease in susceptibility to neutralization by several therapeutic monoclonal antibodies, resulting in substantial loss of clinical efficacy and, consequently, revocation of emergency use authorization for the treatment of COVID-19.⁸

It is known that the additional antigenic exposure from boosters and breakthrough infections bolster serological immunity, and third-dose vaccine booster campaigns are underway worldwide to mitigate the ongoing wave of Omicron cases.^{9,10} Vaccine breakthrough infections can directly train the immune system against variant spike proteins but come with medical risks including prolonged illness (long COVID) and death.^{11,12} Conversely, booster vaccination is generally safe and has been shown to effectively increase the neutralizing response against Omicron BA.1.^{13–15} The durability of responses due to boosting and breakthrough infection are unknown, but antibody levels have been shown to decrease over time following primary vaccination, suggesting that waning of the augmented immunity following additional exposure is likely.^{16,17} It is also unknown whether recovery from breakthrough infection or booster vaccination provide greater protection from reinfection with Omicron sub-variants and any future variants, which will likely affect the future trajectory of the pandemic. To address these knowledge gaps, we examined serological immune responses and antibody-dependent cell-mediated phagocytosis in individuals who had received either two doses of a standard vaccine regimen, a standard regimen followed by a booster, or breakthrough infection following vaccination.

RESULTS

Cohort

A total of 99 individuals were studied (Table 1). Participants from the two-dose group provided serum samples a median of 24 days after the second dose. The three-dose group received a third vaccine dose a median of 253 days after the second and then provided serum samples a median of 86 days after the third dose (Figure 1A). Both two- and three-dose groups reported no history of SARS-CoV-2 infection and displayed a lack of nucleocapsid antibodies (Figure 1B). Breakthrough group participants had infection confirmed by a positive PCR-based COVID-19 test at a median of 159.5 days after their final vaccine dose and provided serum samples a median of 29 days after the date of PCR testing. Among the breakthrough infections, 10 of 30 participants were infected with the Delta variant.

Approach

In each sample, we analyzed the spike RBD-specific immunoglobulin G (IgG), IgA, and IgM antibody levels by enzyme-linked immunosorbent assay (ELISA). We also measured the ability of each serum sample to neutralize authentic wild-type SARS-CoV-2 (WA1) and clinical isolates of the Delta (B.1.617.2) and Omicron (BA.1 and BA.2) variants with focus reduction neutralization tests (FRNTs). Finally, we examined

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Table 1. Cohort demographics and clinical data

Characteristic	Two dose	Three dose	Breakthrough
Cohort characteristics			
N	42	27	30
Female, N (%)	35 (83.3)	19 (70.4)	23 (76.7)
Male, N (%)	7 (16.7)	8 (29.6)	7 (23.3)
Age, year, median (range)	40 (23–74)	47 (26–74)	38 (24–63)
Time intervals, days, median (interquartile range)			
Last vaccine to blood draw	24 (17.25–35.75)	86 (79.5–93.5)	N/A
PCR positivity to blood draw	N/A	N/A	29 (23–47)
Second vaccine to positive PCR	N/A	N/A	159.5 (81.25–202.25)
Time between first and second vaccines	21 (21–22)	21 (21–22)	21 (21–23)
Time between second and third vaccines	N/A	253 (249–263.5)	N/A
Vaccine type, N (%)			
BNT162b2 (Pfizer)	42 (100)	27 (100)	28 (93.3)
mRNA-1237 (Moderna)	0 (0)	0 (0)	1 (3.3)
Ad26.COV2.S (Janssen)	0 (0)	0 (0)	1 (3.3)
Breakthrough infection strain, N (%)			
Alpha (B.1.1.7)	N/A	N/A	5 (16.7)
Beta (B.1.351)	N/A	N/A	1 (3.3)
Gamma (P.1)	N/A	N/A	3 (10)
Delta (B.1.617.2)	N/A	N/A	10 (33.3)
Unknown ^a	N/A	N/A	11 (36.7)

^aAll breakthrough cases occurred between January 1, 2021, and August 18, 2021 (pre-Omicron).

the ability of serum in each group to trigger antibody-dependent cell-mediated phagocytosis (ADCP) of spike protein-coated beads.

Binding antibody responses

Compared with two-dose vaccination, the geometric mean of serum dilutions with half-maximal binding in ELISA (EC_{50}) to full-length SARS-CoV-2 spike protein was 2.23-fold higher in the three-dose group and 2.78-fold higher in the breakthrough group; the three-dose and breakthrough groups were not significantly different from each other (Figure 2A). Spike RBD-specific antibodies did not significantly increase in the three-dose group but did in the breakthrough group, which was 2.8-fold higher than in the two-dose group (Figure 2B). Spike-specific IgG and IgA levels showed similar increases relative to the two-dose group, with 2.2- and 2.5-fold higher IgG levels and 2.2- and 2.9-fold higher IgA levels in the three-dose and breakthrough groups, respectively (Figures 2C and 2D). IgM levels were not significantly different between any of the groups (Figure 2E).

Antibody-dependent cell-mediated phagocytosis

Similar to neutralizing antibody responses, ADCP also increased in the three-dose (1.8-fold) and breakthrough (2.2-fold) groups compared with two-dose vaccination; here, as well, the three-dose and breakthrough groups were not significantly different from each other (Figure 2F).

Neutralizing antibody responses

Consistent with previous reports, neutralization of live SARS-CoV-2 improved to a greater degree than the observed rise in binding antibody levels.^{10,18} The geometric mean titers (GMTs) showing 50% neutralization of the original SARS-CoV-2 virus (WA1) in FRNT assays were 4.6- and 7.1-fold higher for the three-dose and breakthrough groups, respectively, compared with two-dose vaccination, but were not significantly different from each other (Figure 3A). The GMT of the breakthrough

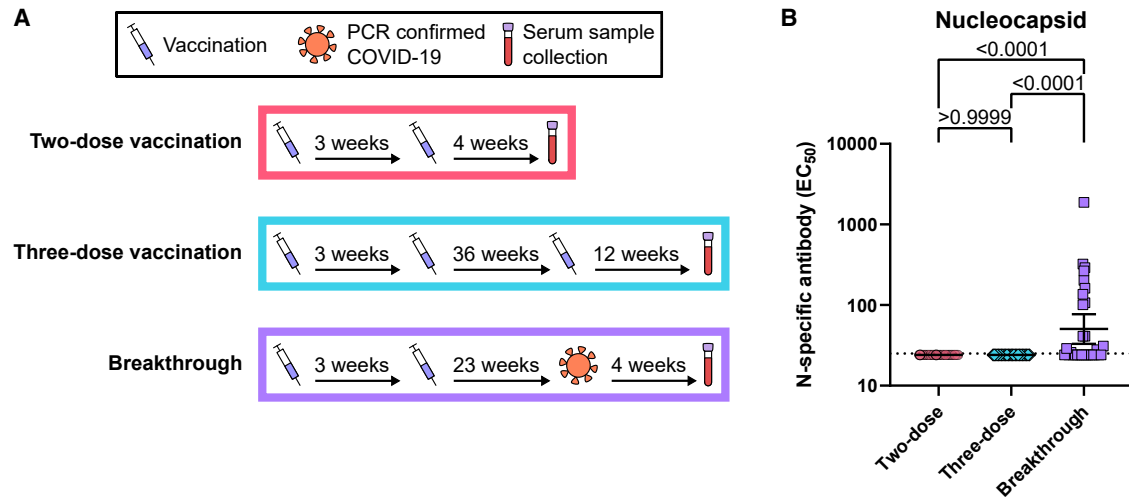


Figure 1. Cohort design

(A) Schematic describing median cohort vaccine dose, PCR-confirmed natural infection, and sample collection timing.

(B) Serum dilutions with half-maximal binding (EC_{50}) of IgG/A/M antibodies to SARS-CoV-2 nucleocapsid protein.

Error bars indicate the geometric mean and 95% confidence intervals. p values show the results of a two-tailed Kruskal-Wallis test with Dunn's multiple comparison correction.

group to neutralize the Delta variant increased 9.0-fold, while the three-dose group increased only 4.2-fold, compared with two-dose vaccination. However, the difference between the three-dose and breakthrough groups did not rise to the level of statistical significance (Figure 3B). Against Omicron BA.1, 25 of 42 (59%) sera in the two-dose group fell below the limit of detection for neutralization, while all three-dose and breakthrough participants showed detectable neutralization, with 11.6- and 16.8-fold higher neutralizing GMTs, respectively, which were not significantly different from each other (Figure 3C). Against Omicron BA.2, 50% of sera in the two-dose group fell below the limit of detection, while all three-dose and breakthrough participants showed detectable neutralization with 5.4- and 12.3-fold higher GMTs than the two-dose group, respectively, but were not significantly different from each other (Figure 3D).

Antibody response quality

The relationship between spike-binding antibody level and neutralizing titer gives an indication of the quality of the antibody response by controlling for the total quantity of antibodies present. In all three groups, binding antibody titer correlated strongly with neutralization of WA1 and Delta. However, the correlations were weaker for the two-dose group against Omicron BA.1 and BA.2, largely due to the high proportion of samples below the detection limit (Figures 3E–3H). We explored this association further by calculating the neutralizing potency index (NPI) as the ratio of live-virus neutralization to spike-specific antibody EC_{50} for WA1, Delta, Omicron BA.1, and Omicron BA.2. For WA1, the median NPI was 0.60 for two dose, 1.10 for three dose, and 1.91 for breakthrough, showing an increase in the ratio of neutralizing activity to spike-binding EC_{50} (Figure 3I). The median Delta NPI was 0.30, 0.52, and 0.94; the median Omicron BA.1 NPI was 0.03, 0.15, and 0.20; and the median Omicron BA.2 NPI was 0.05, 0.12, and 0.14 for the two-dose, three-dose, and breakthrough groups, respectively (Figures 3J–3L). NPI values were significantly increased in the three-dose and breakthrough groups relative to the two-dose group for all viruses tested but were not significantly different from each other.

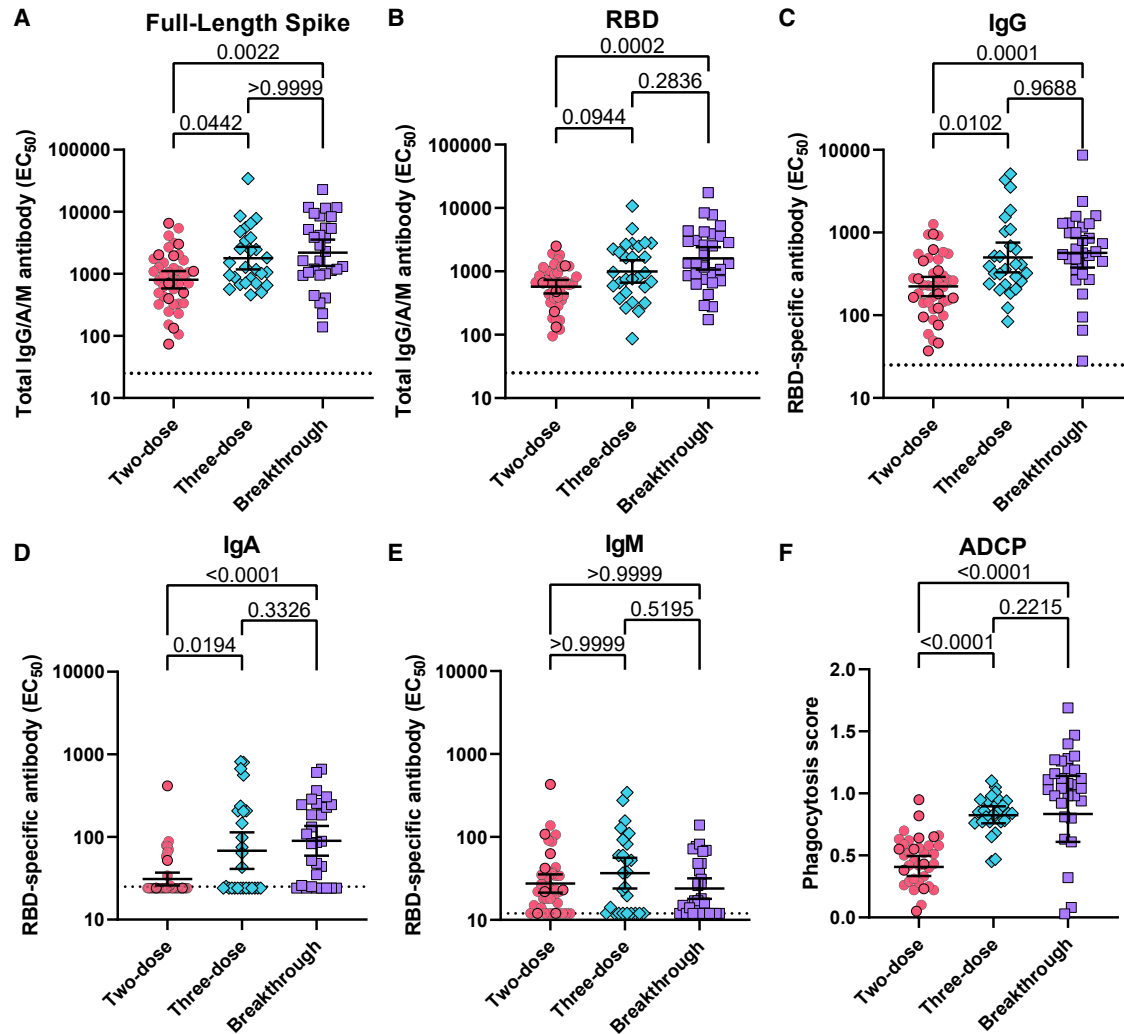


Figure 2. Antibody response to two-dose vaccination, three-dose vaccination, and breakthrough infection

(A) Serum dilutions with EC₅₀ of IgG/A/M antibodies to full-length SARS-CoV-2 spike protein.

(B) Serum IgG/A/M antibody EC₅₀ to spike receptor-binding domain (RBD).

(C–E) Serum (C) IgG-, (D) IgA-, and (E) IgM-specific antibody EC₅₀s to RBD.

(F) Antibody-dependent cellular phagocytosis scores indicate the increase in uptake of RBD-coated beads caused by sera.

Error bars indicate the geometric mean and 95% confidence intervals. p values show the results of two-tailed Kruskal-Wallis tests with Dunn's multiple comparison correction.

A similar trend was seen when calculating the NPI using RBD-specific antibody levels instead of those for full-length spike (Figures 3M–3P).

Relative loss of strain-specific neutralizing capacity

Comparing the neutralization of Delta and Omicron BA.1 and BA.2 with that of WA1 clearly showed a greater extent of resistance by Omicron sub-variants, with some individuals displaying a nearly 100-fold reduction in neutralization of Omicron compared with WA1 (Figures 4A–4C). To quantify the relative loss of neutralizing activity against the Delta and Omicron (BA.1 and BA.2) variants compared with WA1, we calculated the ratio of neutralization for each variant to WA1 neutralization in each participant. For Delta, the median ratio was 0.42 for two dose, 0.43 for three dose, and 0.55 for breakthrough, none of which were significantly different (Figure 4D). Against Omicron BA.1, however, the median ratio was 0.06 for two dose,

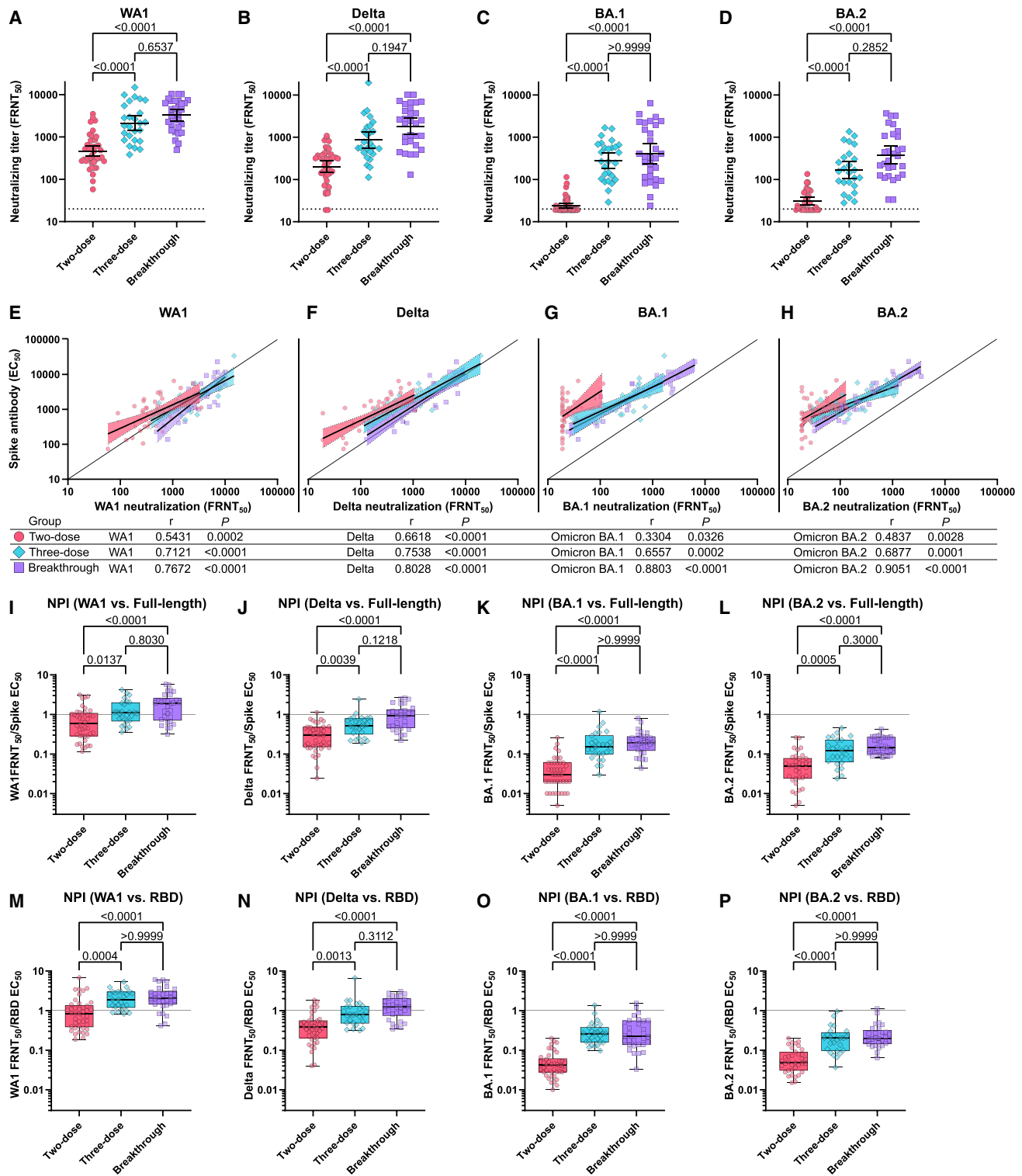


Figure 3. Live SARS-CoV-2 neutralization by two-dose vaccination, three-dose vaccination, and breakthrough infection cohorts
 (A–D) Wild-type SARS-CoV-2 (WA1) (A), Delta (B), Omicron BA.1 (C), and Omicron BA.2 (D) neutralizing activity determined by 50% focus reduction neutralization test (FRNT₅₀).
 (E–H) Correlation of serum full-length spike-binding antibody EC₅₀ with (E) WA1 FRNT₅₀, (F) Delta FRNT₅₀, (G) Omicron BA.1 FRNT₅₀, and (H) Omicron BA.2 FRNT₅₀. The solid line indicates equal EC₅₀ and FRNT₅₀ values.

Figure 3. Continued

(I–L) Neutralizing potency indexes show the ratio of (I) WA1, (J) Delta, (K) Omicron BA.1, and (L) Omicron BA.2 FRNT₅₀ over full-length spike-specific antibody EC₅₀.

(M–P) Neutralizing potency indexes calculated instead with RBD-specific antibody EC_{50s} for (M) WA1, (N) Delta, (O) Omicron BA.1, and (P) Omicron BA.2. The solid gray lines indicate equal FRNT₅₀ and EC₅₀.

Error bars in (A)–(D) indicate the geometric mean and 95% confidence intervals. Box plots in (I)–(P) show the median, interquartile range, and full range. *p* values in (A)–(D) and (I)–(P) show the results of two-tailed Kruskal-Wallis tests with Dunn's multiple comparison correction.

0.12 for three dose, and 0.13 for breakthrough, and here, the three-dose and breakthrough groups were significantly higher than the two-dose group. The corresponding Omicron BA.2 median ratios were 0.07, 0.10, and 0.10, respectively, and no groups were significantly different from each other (Figures 4E and 4F).

Antibody response versus age and gender

Previous studies have established a negative correlation between antibody response and age among vaccinated individuals.¹⁹ Among study participants, we observed a negative correlation between age and WA1 neutralizing titer for the two-dose and also the three-dose groups but not for the breakthrough group (Figure 4G). We then calculated the correlation between age and neutralizing titer against WA1 as well as Delta, Omicron BA.1, and Omicron BA.2, which is depicted in a heatmap (Figures 4H and S1A–S1C). We found no difference in neutralizing titer based on gender (Figures S2A–S2D).

DISCUSSION

We compared SARS-CoV-2-specific serological and sero-dependent immune responses in individuals receiving two standard vaccine doses and three vaccine doses and individuals experiencing breakthrough infection. We found that despite the reliance of the vaccine on the original SARS-CoV-2 spike protein sequence, both booster vaccination and breakthrough infection enhance serological responses to a similar degree. In each of these re-exposure groups, we observed significant increases in IgG binding levels, antibody-dependent cell-mediated phagocytosis, and neutralizing titers. Further, we observed improved breadth of the humoral response, as seen by improved Delta and Omicron BA.1 and BA.2 variant neutralization and an increased ratio of variant to WA1 neutralizing titers, and improved antibody quality, as reflected in an improvement in the amount of neutralizing activity for a given spike-binding antibody titer.

Thus, while two doses of the currently available mRNA vaccines provide robust antibody responses correlating with strong protection against symptomatic infection due to the original SARS-CoV-2 and early variants, serological immunity against the Omicron BA.1 and BA.2 variants is substantially reduced²⁰ but is restored both by booster vaccination and breakthrough infection. This is consistent with a previous study indicating that hybrid immunity from SARS-CoV-2 infection followed by one or two mRNA vaccine doses provides similar antibody responses to breakthrough infection.¹⁰ Interestingly, the negative correlation between age and antibody levels seen in those exposed through vaccination alone is not seen in those who have experienced breakthrough infection.

Despite the augmented immune responses seen with additional exposure after receipt of a primary series, our data also highlight a progressive loss of susceptibility to neutralizing responses with the emergence of new variants, consistent with recent reports.^{2,4} This is most clearly evident in the declining NPI (degree of neutralization for a given amount of antibody), the declining ratio of variant neutralization to WA1

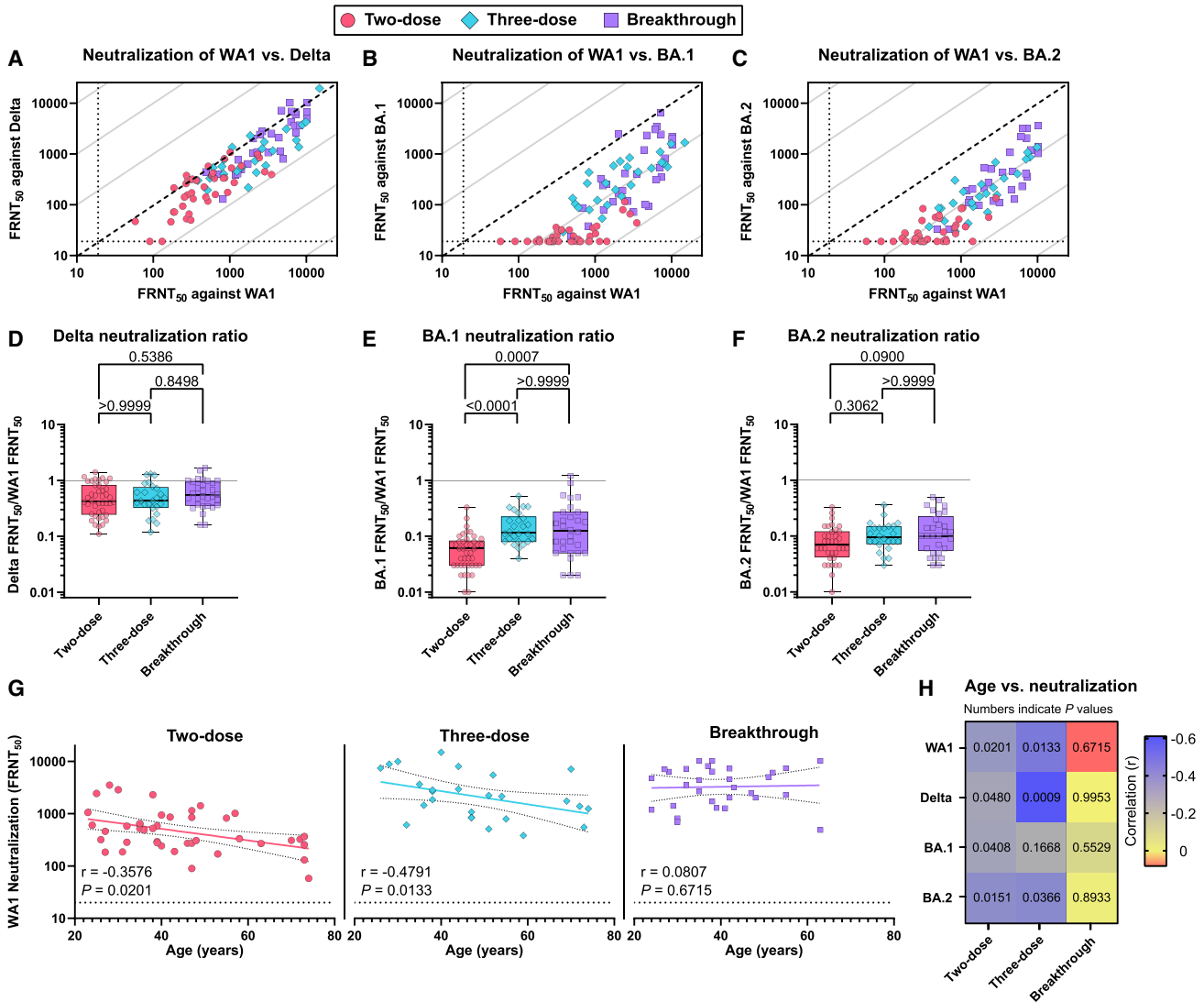


Figure 4. Quality of the neutralizing antibody response to two-dose vaccination, three-dose vaccination, and breakthrough infection (A–C) Scatter plots depicting (A) Delta, (B) Omicron BA.1, and (C) Omicron BA.2 FRNT₅₀ versus WA1 FRNT₅₀. The broken lines indicate equal neutralization of variants and WA1, while gray lines signify 10-fold differences. (D–F) Relative neutralization of (D) Delta, (E) Omicron BA.1, and (F) Omicron BA.2 FRNT₅₀ over WA1 FRNT₅₀. The solid line indicates equal neutralization of WA1 and variants. (G) Correlation of WA1 FRNT₅₀ with age at the time of study enrollment. (H) Heatmap showing correlation between age and FRNT₅₀ for WA1, Delta, Omicron BA.1, and Omicron BA.2 for two-dose, three-dose, and breakthrough groups.

Box plots in (D)–(F) show the median, interquartile range, and full range, while p values show the results of two-tailed Kruskal-Wallis tests with Dunn’s multiple comparison correction. r values in (G) indicate the Spearman correlation coefficients with corresponding two-tailed p values. Linear best-fit lines with 95% confidence bands were determined by simple linear regression of log-transformed FRNT₅₀ and non-transformed age values. Colors in (H) indicate Spearman correlation coefficients, and values indicate the corresponding p values. See also [Figure S1](#).

neutralization in the progression from Delta to the Omicron sub-variants, and subtle differences in this ratio suggesting further loss between BA1 and BA2.

The similarity seen between immune responses to three-dose regimens and breakthrough infection suggests that vaccines based on the original WA1 variant continue to provide neutralizing antibody responses conferring at least partial protection against currently circulating variants, including early Omicron sub-lineages.

However, the progressive loss in neutralizing capacity suggests that boosting with updated vaccine inserts will likely take on an important role in developing effective prophylaxis against future SARS-CoV-2 variants. Both Pfizer and Moderna have both recently pursued bivalent vaccine approaches; Moderna has studied a bivalent vaccine containing inserts corresponding to the original strain and B.1.351, and both Pfizer and Moderna are developing similar vaccines pairing original-strain and Omicron-adapted inserts.^{21–23} While reports suggest improved immunogenicity for each of these, peer-reviewed data are not yet available, and doubts remain about the relevance of these vaccines to the emerging BA.4/5 sub-variants and anticipated future variants.²⁴

This debate highlights the inherent difficulty in evaluating immune responses to vaccination in the face of such a highly prevalent and rapidly evolving viral pandemic, since the emergence of new strains continually challenges our current understanding of the minimum requirements for a broadly effective vaccine. While we provide additional data on early Omicron variants, ongoing work will be required to understand the impact of BA.4/5 and future lineages and whether periodic vaccine updates, multivalent vaccines, or perhaps vaccines focusing on relatively invariant portions of the spike protein will be the key to finding an effective long-term vaccine strategy against SARS-CoV-2.

Limitations of the study

One limitation of this study is the slightly longer time to sampling in the three-dose group compared with the other groups. The protection from three-dose vaccination is known to decrease measurably over the first 3 months,²⁵ and therefore neutralizing antibody levels may be underestimated in our data relative to the other groups. In addition, age distributions were not perfectly matched in the three groups studied, though the trends we observed persisted even in a sub-analysis of those <65 years of age (Figure S3). Lastly, participants were recruited from among healthcare workers self-reporting infection or otherwise volunteering for the study, and it was not possible to recruit a cohort broadly representative of diverse ethnic groups.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.medj.2022.09.001>.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.E.C., F.G.T., and T.A.B.; recruitment and sample collection, M.E.C., D.S., and S.D.C.; experimental design, M.E.C., F.G.T., and T.A.B.; laboratory analysis, F.G.T., T.A.B., G.G., and S.K.M.; statistical analysis, T.A.B.; supervision: M.E.C. and F.G.T.; manuscript drafting, M.E.C. and T.A.B.; manuscript review and editing, M.E.C., T.A.B., D.S., S.D.C., and F.G.T.

DECLARATION OF INTERESTS

The authors report no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-human IgA-HRP	BioLegend	411002
Mouse anti-human IgG-HRP Clone G18-145	BD Biosciences	555788
Goat anti-human IgM-HRP	Bethyl Laboratories	A80-100P
anti-SARS-CoV-2 alpaca serum	Capralogics Inc.	Custom
anti-alpaca-HRP	Novus	NB7242
Bacterial and virus strains		
USA-WA1/2020 [lineage A]	BEI Resources	NR-52281
hCoV-19/USA/PHC658/2021 [lineage B.1.617.2 – Delta]	BEI Resources	NR-55611
hCoV-19/USA/MD-HP20874/2021 [lineage B.1.1.529 – Omicron BA.1]	BEI Resources	NR-56461
hCoV-19/USA/CO-CDPHE-2102544747/2021 [lineage B.1.1.529 – Omicron BA.2]	BEI Resources	NR-56520
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 Spike RBD	In house	N/A
SARS-CoV-2 Nucleocapsid-His	BEI Resources	NR-53797
o-phenylenediamine dihydrochloride (OPD)	Thermo Scientific	34005
TrueBlue	Sera Care	5510-0030
Experimental models: Cell lines		
Expi293F cells	ThermoFisher	A14527
THP-1 cells	ATCC	TIB-202
Vero E6 cells	ATCC	CRL-1586
Software and algorithms		
Prism version 9.3.1	Graphpad	https://www.graphpad.com/scientific-software/prism/
Immunospot analyzer v5.3	CTL	https://immunospot.com/products/analyzers
Viridot focus counting package for R v1.0	Katzelnick et al., 2018	https://github.com/leahkatzelnick/Viridot
R version 4.1.0	R Project	https://cran.r-project.org/
Dose response calculator	Bates et al., 2021	https://doi.org/10.5281/zenodo.5158655
Other		
EIA/RIA high binding ELISA plates	Corning	Ref #359096
Neutravidin beads	Invitrogen	F8775

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Dr. Marcel Curlin (curlin@ohsu.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Neutralizing antibody titers and all other assay data reported in this paper will be shared by the [lead contact](#) upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cohort selection and serum collection

Two- and three-dose group participants were selected from a larger cohort of vaccinated health care workers at Oregon Health & Science University recruited at the time of their first vaccine dose. Participants were asked to return after either their second or third vaccine dose to provide whole blood samples. Breakthrough group participants were recruited and enrolled at Oregon Health & Science University from among fully vaccinated health care workers receiving positive results during PCR-based diagnostic testing for SARS-CoV-2 infection, at which time participants provided information on symptoms of illness by direct interview. Whole blood (4–6 mL) was collected with a BD Vacutainer® Plus Plastic Serum Tube and centrifuged for 10 min at 1000xg, then stored at –20°C. Two- and three-dose group participants confirmed no history of COVID-19 by direct interview and validated by nonreactivity in a SARS-CoV-2 N protein ELISA. The vaccines used in this study were BNT162b2 (Pfizer), mRNA-1273 (Moderna), or Ad26.COV2.S (Janssen) and only individuals with no reported immunocompromising conditions were included. Participants information on sex, and age, was self-reported. Information on race, gender and socioeconomic status was not collected.

Ethics statement

This study was conducted with approval of the Oregon Health and Sciences University Institutional review board (IRB# 00022511). All participants were enrolled following written informed consent.

METHOD DETAILS

Enzyme-linked immunosorbent assays (ELISA)

ELISAs were performed as previously described.¹⁹ In 96-well ELISA plates. Plates were coated with SARS-CoV-2 RBD (produced in Expi293F cells and purified using Ni-NTA chromatography), N at 100 µL/well at 1 µg/mL in PBS and incubated overnight at 4°C with rocking. Plates were washed three times with 0.05% Tween 20 in PBS (wash buffer) and blocked with 150 µL/well with 5% nonfat dry milk powder and 0.05% Tween 20 in PBS (blocking buffer) at room temperature (RT) for 1 h with rocking. Breakthrough and control sera were aliquoted and frozen in dilution plates then resuspended in blocking buffer; sera were diluted and added to ELISA plates 100 µL/well (6 x 4-fold dilutions from 1:50 to 1:51,200, except for IgM (6 x 4-fold dilutions from 1:25 to 25,600). Sera was incubated in coated plates for 1 h at RT, then washed three times with wash buffer. Plates were incubated with anti-human IgA-HRP at 1:3,000, Mouse anti-human IgG-HRP at 1:3,000, or Goat anti-human IgM-HRP at 1:3,000 at RT for 1 h with rocking, then washed three times with wash buffer prior to developing with o-phenylenediamine dihydrochloride (OPD) according to manufacturer instructions. The reaction was stopped after 25 min using an equivalent volume of 1 M HCl; optical density was measured at 492 nm using a CLARIOstar plate reader.

Antibody dependent cellular phagocytosis (ADCP)

ADCP was assessed as described previously.¹⁰ Biotinylated SARS-CoV-2 RBD protein was incubated with neutravidin beads for 2 h at room temperature then washed with PBS with 1% BSA (dilution buffer) two times. 10 µL of 1:100 diluted RBD beads were incubated with an equal volume of diluted serum for 2 h at 37°C. Bead-serum mixtures were then incubated with 20,000 THP-1 cells in a final volume of 100 µL overnight in a tissue culture incubator. 100 µL of PBS with 4% formaldehyde was then used to fix each well for 30 min prior to flow cytometry. Triplicate, samples

were flowed on a CytoFLEX flow cytometer. 2500 events were recorded per replicate. Phagocytosis scores were calculated as the product of percent bead-positive cells and mean fluorescence intensity of bead-positive cells, then divided by 10^6 .

SARS-CoV-2 growth and titration

SARS-CoV-2 isolates USA-WA1/2020 [lineage A], hCoV-19/USA/PHC658/2021 [lineage B.1.617.2 – Delta], hCoV-19/USA/MD-HP20874/2021 [lineage B.1.1.529 – Omicron BA.1], and hCoV-19/USA/CO-CDPHE-2102544747/2021 [lineage B.1.1.529 – Omicron BA.2] were obtained from BEI Resources. Viral stocks were propagated as previously described.²⁶ Sub-confluent Vero E6 cells grown in Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% penicillin-streptomycin (complete media) were infected at an MOI of 0.05 in a minimal volume (0.01 mL/cm²) of Opti-MEM + 2% FBS (dilution media) for 1 h at TCC then 0.1 mL/cm² additional complete media was added and incubated until at least 20% cytopathic effect (CPE) was observed, typically 72–96 h. Culture supernatant was centrifuged for 10 min at 1000xg and frozen at -80°C . Titration was performed by focus forming assay on sub-confluent Vero E6 cells. 10-fold dilutions were prepared in dilution media and incubated for 1 h, then covered with Opti-MEM, 2% FBS, 1% methylcellulose (overlay media) and incubated for 24 h (48 h for Omicron BA.1 and BA.2). Plates were then fixed in 4% formaldehyde in phosphate buffered saline (PBS) for 1 h then removed from BSL-3 following institutional guidelines. Cells were permeabilized in 0.1% bovine serum albumin (BSA), 0.1% saponin in PBS (perm buffer) for 30 min, and were then incubated with polyclonal anti-SARS-CoV-2 alpaca serum (1:5000 in perm buffer, or 1:2000 for Omicron) overnight at 4°C . Plates were washed three times with 0.01% Tween 20 in PBS (wash buffer), then incubated for 2 h at RT with 1:20,000 anti-alpaca-HRP, or 1:5000 for Omicron. Plates were washed three times with wash buffer, then incubated with TrueBlue for 30 min or until sufficiently developed for imaging. Foci images were captured with a CTL Immunospot Analyzer and counted with Viridot (1.0) in R (3.6.3).²⁷ Viral stock titers in focus forming units (FFU) were calculated based on the dilution factor and volume used for infection.

Focus reduction neutralization test (FRNT)

FRNT assays were carried out as previously described.²⁶ We prepared 5×4.7 -fold (1:10–1:4879) serial dilutions in duplicate for each serum sample. An equal volume of viral stock was added to each well (final dilutions of sera, 1:20–1:9760) such that approximately 50 FFU were added to each well. Virus-serum mixtures were incubated for 1 h before being used to infect sub-confluent Vero E6 cells in 96-well plates for 1 h, then covering with 150 μL /well overlay media. Each 5-point serum dilution series was accompanied by a virus only control well. Fixation, development, and counting of FRNT plates was carried out as described in SARS-CoV-2 growth and titration. Percent neutralization values were calculated for each well as focus count divided by the average of virus-only wells from the same plate.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

FRNT₅₀ and EC₅₀ values were calculated by fitting to a dose-response curve as previously described.²⁶ Final FRNT₅₀ values below the limit of detection (1:20) were set to 1:19. Final EC₅₀ values below the limit of detection of 1:25 for N, full-length Spike, Spike RBD, IgG, IgA were set to 1:24 and 1:12.5 for IgM was set to 1:12. Aggregated EC₅₀ and FRNT₅₀ values were analyzed in Graphpad Prism (9.3.1). Significance was determined using Kruskal-Wallis tests with Dunn's multiple comparison correction, p-values were two-tailed. Correlations were calculated with log-transformed EC₅₀ and/or FRNT₅₀ values with the Spearman method, with corresponding two-tailed p values. Best fit lines were calculated via simple linear regression.