CORONAVIRUS

Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants

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Current coronavirus disease 2019 (COVID-19) vaccines effectively reduce overall morbidity and mortality and are vitally important to controlling the pandemic. Individuals who previously recovered from COVID-19 have enhanced immune responses after vaccination (hybrid immunity) compared with their naïve-vaccinated peers; however, the effects of post-vaccination breakthrough infections on humoral immune response remain to be determined. Here, we measure neutralizing antibody responses from 104 vaccinated individuals, including those with breakthrough infections, hybrid immunity, and no infection history. We find that human immune sera after breakthrough infection and vaccination after natural infection broadly neutralize SARS-CoV-2 (severe acute respiratory coronavirus 2) variants to a similar degree. Although age negatively correlates with antibody response after vaccination alone, no correlation with age was found in breakthrough or hybrid immune groups. Together, our data suggest that the additional antigen exposure from natural infection substantially boosts the quantity, quality, and breadth of humoral immune response regardless of whether it occurs before or after vaccination.

INTRODUCTION

Severe acute respiratory coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic. Globally, cases continue to increase despite worldwide vaccination campaigns (1). Numerous safe and effective vaccines have been developed that effectively reduce the risk of infection, severe disease, and death including BNT162b2 (Pfizer), mRNA-1273 (Moderna), and Ad26.COV2.S (Janssen) (2, 3). However, variants of concern (VOCs) with differing levels of increased transmissibility and resistance to existing immunity have sequentially emerged, spread widely, and receded over time since the beginning of the pandemic (4–7). Several studies have shown that antibody responses from the initial wave of vaccines in early 2021 have waned over the 6 months after vaccination, possibly contributing to an increase in breakthrough infections (8–12). Booster vaccine doses were first approved in Israel in July 2021 and have since been more widely adopted in other countries to address these concerns despite the concern that booster campaigns may divert much needed vaccine doses away from lower-income countries (13).

Vaccination after recovery from natural SARS-CoV-2 infection, or "hybrid immunity," has been reported to substantially increase both the potency and breadth of humoral response to SARS-CoV-2 (14, 15). However, current studies on breakthrough infection occurring after vaccination have focused on identifying susceptibility factors such as virus neutralizing titer before infection (16). The impact of breakthrough infection on the neutralizing antibody response and how this compares with the response elicited by hybrid immunity remains unclear; we therefore undertook the present study to directly address this gap in knowledge.

RESULTS

Cohort and study design

We recruited a total of 104 participants (Table 1) consisting of 31 fully vaccinated individuals with polymerase chain reaction (PCR)–confirmed breakthrough infections, 31 individuals with one (6 individuals) or two vaccine (25 individuals) doses after recovery from COVID-19 (hybrid immunity), and 42 fully vaccinated individuals with no history of COVID-19 or breakthrough infection (Fig. 1A). Ninety-six participants received BNT162b2, six received mRNA-1273, and two received Ad26.COV2.S. Serum samples were collected from each of the participants, which were then tested for 50% effective antibody concentrations (EC$_{50}$) by enzyme-linked immunosorbent assay (ELISA) and 50% live SARS-CoV-2 neutralizing titer with focus reduction neutralization tests (FRNT$_{50}$) against early lineage strain SARS-CoV-2 (WA1) and clinical isolates of three VOCs: Alpha (B.1.1.7), Beta, (B.1.351), and Delta (B.1.617.2). We performed additional antibody-dependent cellular phagocytosis (ADCP) experiments to evaluate any functional differences in the antibody response of each group.

We first analyzed the hybrid immunity of participants who received only a single vaccine dose compared with those who had received two doses (fig. S1). All measures of antibody levels, ADCP, and live virus neutralization revealed no significant difference between these two groups. For this reason, we combined these samples into a single group containing participants with both one and two vaccine doses after natural infection, which we henceforth refer to as the hybrid immune group.

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Table 1. Cohort demographics. IQR, interquartile range; N/A, not applicable.

Characteristic		Vaccine only	Hybrid immunity	Breakthrough
		N = 42	*N* = 31	*N* = 31
Sex				
	Female—*N* (%)	35 (83.3)	19 (61.3)	24 (77.4)
	Male—*N* (%)	7 (16.7)	12 (38.7)	7 (22.6)
Age (years)				
	Median [range]	40 [23–74]	50 [23–73]	38 [24–63]
Critical time periods (days)—median [IQR]				
	Latest vaccine dose to blood draw	24 [17.25–35.75]	25 [17.5–34]	N/A
	PCR positivity to blood draw	N/A	N/A	35 [23–48.5]
	PCR positivity to first vaccine dose	N/A	289 [124–334.5]	N/A
	Second vaccine dose to PCR-positive	N/A	N/A	139 [81.5–201.5]
	Days between vaccine doses	21 [21–22]	22 [21–25]	21 [21–23]
Vaccine type—*N* (%)				
	BNT162b2 (Pfizer)	42 (100)	25 (80.6)	29 (93.5)
	mRNA-1273 (Moderna)	0 (0)	5 (16.1)	1 (3.2)
	Ad26.COV2.S (Janssen)	0 (0)	1 (3.2)	1 (3.2)

Antibody levels after breakthrough infection, hybrid immunity, and vaccination alone

ELISA geometric mean titer (GMT) EC_{50} values for SARS-CoV-2 spike-specific antibodies were significantly elevated in both the breakthrough (2.5-fold, $P = 0.005$) and hybrid immune (3.6-fold, $P < 0.0001$) groups compared with vaccination alone, but we saw no significant difference between the breakthrough and hybrid groups (Fig. 1B). A similar trend was seen for EC_{50} values specific for the spike receptor-binding domain (RBD) (Fig. 1B). We additionally confirmed that none of the vaccine-only participants exhibited reactivity against the nucleocapsid (N) protein, supporting lack of previous infection, whereas the breakthrough and hybrid immune groups were 68 and 48% N-responsive, respectively (Fig. 1B). Opsonization with hybrid immune and breakthrough sera also induced phagocytosis of spike protein–coated particles in an ADCP assay significantly more than vaccination alone but not compared with each other (Fig. 1C). The levels of immunoglobulin G (IgG) and IgA antibodies specific to RBD protein displayed a similar trend to the total EC_{50} levels with significant increases for hybrid immunity and breakthrough compared with vaccination alone but not compared with each other (Fig. 1D). RBD-specific IgM values were notably low and did not differ significantly between groups. Consistent with previous reports (*17*), spike-specific antibody levels correlated negatively with age among vaccine-only participants. In contrast, neither the breakthrough nor hybrid immune group recapitulated this correlation, displaying no significant age-related trend (Fig. 1E).

Neutralizing antibody titers against SARS-CoV-2 and the VOCs

We next quantified the functional activity of participants' immune sera by comparing their neutralization titers against early (WA1) SARS-CoV-2 and selected VOCs. Against all viruses, the trend mirrored that of the antibody EC_{50} levels, with the vaccine-only group $FRNT_{50}$ titers significantly lower than both breakthrough and hybrid immunity, which were comparable with each other (Fig. 2A). The $FRNT_{50}$ GMTs of hybrid immune group participants were 10.8-, 16.9-, 32.8-, and 15.7-fold higher than vaccination alone for WA1, Alpha, Beta, and Delta variants, respectively, whereas breakthrough group participants were 6.0-, 11.8-, 17.0-, and 8.5-fold higher than vaccination alone, respectively, all with $P < 0.0001$. Among vaccine group participants, neutralization of the Beta variant was significantly reduced compared with WA1, whereas the difference seen for the hybrid immune and breakthrough groups was not significant (fig. S2).

In addition to eliciting immunity with greater breadth (Fig. 2A), the serum antibody potency across the breadth of VOCs tested was greater for both hybrid immune and breakthrough groups, as measured by an increase in the ratio of variant neutralization over WA1 $FRNT_{50}$ values against Alpha and Beta for the hybrid immune and breakthrough groups and against Delta for the hybrid immune group (Fig. 2B and fig. S3). Breakthrough and hybrid immune participants grouped more tightly and displayed variant neutralizing titers closer to that of WA1 (Fig. 2, C to E).

Quality of the neutralizing antibody response

We also found that hybrid immunity was associated with a remarkable improvement in the proportion of spike-specific antibodies that were also neutralizing. WA1 neutralizing titers correlated with spike-specific antibody levels for all three groups, but the hybrid immune and breakthrough groups correlated more strongly (Fig. 3A). To analyze the efficiency of sera at neutralizing a given virus strain, we determined a neutralizing potency index by calculating the ratio of
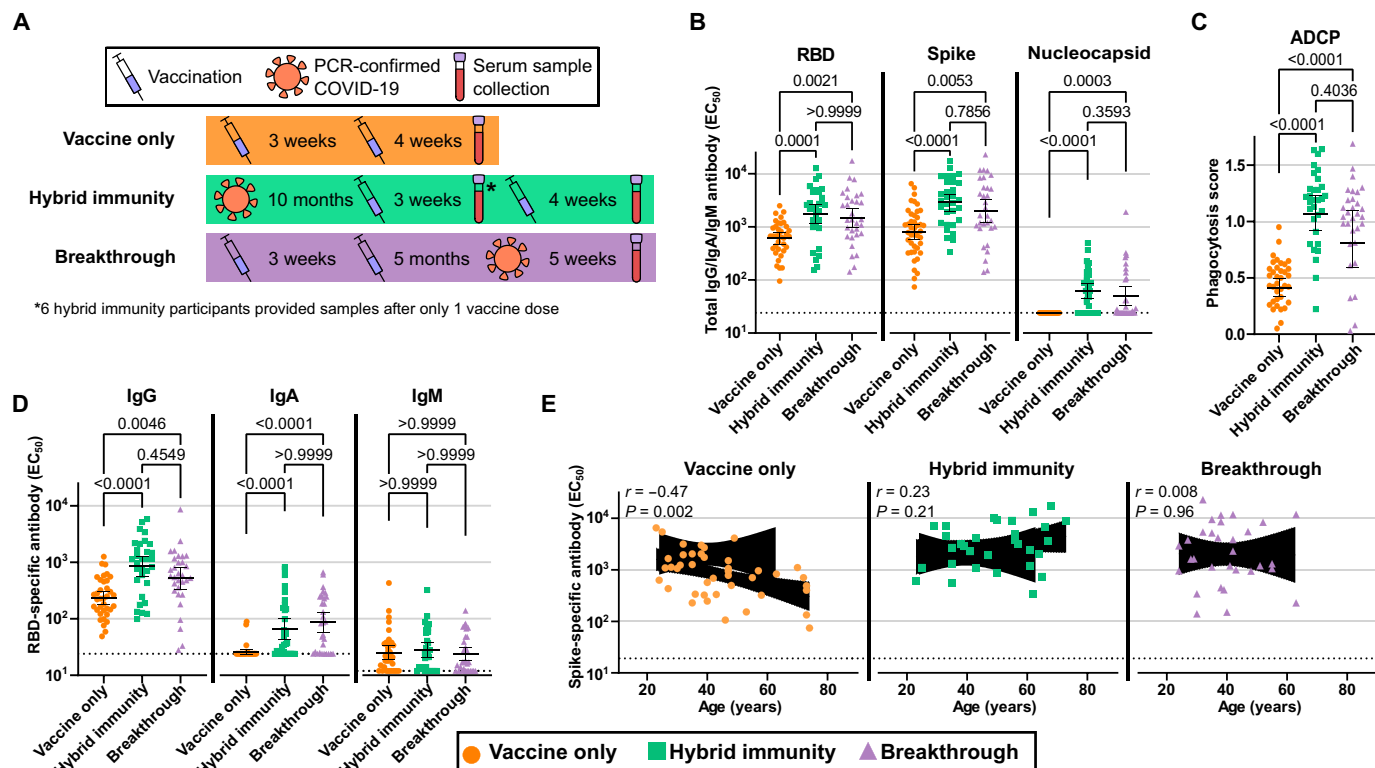
Fig. 1. Antibody levels after breakthrough infection, hybrid immunity, and vaccination alone. (**A**) Schematic depicting the order and approximate time scale of vaccination and natural infection for each group. The blue syringe indicates a dose of vaccine, the orange virus particle indicates PCR-confirmed natural infection with SARS-CoV-2, and the purple-capped vial indicates serum collection. The asterisk (*) indicates that 6 (of 31) hybrid immune participants provided serum samples after only a single vaccine dose. (**B**) IgG/IgA/IgM inverse fold-dilution EC_{50} values for sera specific to RBD, full-length spike, and nucleocapsid proteins measured by ELISA. (**C**) ADCP scores. (**D**) RBD-specific EC_{50} values for IgG, IgA, and IgM class antibodies measured by ELISA. (**E**) Correlation between spike-specific EC_{50} values and participant age. Error bars in (B) and (D) indicate the geometric mean with the 95% confidence interval, whereas error bars in (C) indicate the arithmetic mean with the 95% confidence interval. P values in (B) to (D) were calculated with two-tailed Kruskal-Wallis test with Dunn's multiple comparison correction. Scatterplots in (E) depict the simple linear fit of age and log-transformed EC_{50} values with 95% confidence bands along with the Spearman's rank correlation coefficient and two-tailed P value.

neutralizing titer ($FRNT_{50}$) to spike binding EC_{50} values (*18*). The index expresses a ratio of fold-serum-dilution with 50% neutralization potency to fold-serum-dilution 50% spike binding capacity or a relative neutralizing antibody–to–total antibody ratio for a given subject's serum. The neutralizing potency index was significantly higher among hybrid immune and breakthrough participants than after vaccination alone (Fig. 3B). Last, we found that the relationship between age and total antibody levels also extends to neutralizing titer; vaccine-only participants displayed a clear negative correlation with age, whereas the hybrid immune and breakthrough participants showed no such correlation (Fig. 3C). No association was seen between reported sex and neutralizing titer for any of the groups (Fig. 3D).

DISCUSSION

Overall, our results show that SARS-CoV-2 infection before or after vaccination gives a significantly larger boost to the neutralizing antibody response compared with two doses of vaccine alone. The potency and breadth of the antibody response appear to improve concomitantly. It has been well established that natural infection alone provides short-lived protection from infection (*17*), showing the importance of vaccination, regardless of infection history. Because vaccination protects against severe disease and death (*19*), it is

safer for individuals to be vaccinated before rather than after natural infection.

The negative correlation between age and neutralizing antibody levels after vaccination alone is an effect that has been previously identified (*20*). The relationship between age and antibody levels after natural infection is markedly more complex, with a peak in antibody levels seen between the ages of 60 and 80 (*21*). The exact reasons for this association remain to be determined, but one hypothesis is that the greater disease severity among individuals of advanced age leads to an overall greater humoral response (*18*). These two opposing trends may obscure any age dependence of antibody levels in the present study among patients with humoral responses resulting from both vaccination and natural infection.

Recent studies have suggested that the humoral response continues to develop long after vaccination, with memory B cells at late time points after vaccination showing improved quality and breadth compared with early time points (*14*, *15*, *22*). Our data cannot separate the contribution of mixed boosting due to the combination of vaccination with natural infection from the contribution of ongoing memory B cell development during the time between first antigen exposure and most recent boosting, whether from vaccination or breakthrough infection. Future studies with individuals who have been vaccinated and boosted may be able to distinguish between these possibilities, and an early study suggests that booster vaccination
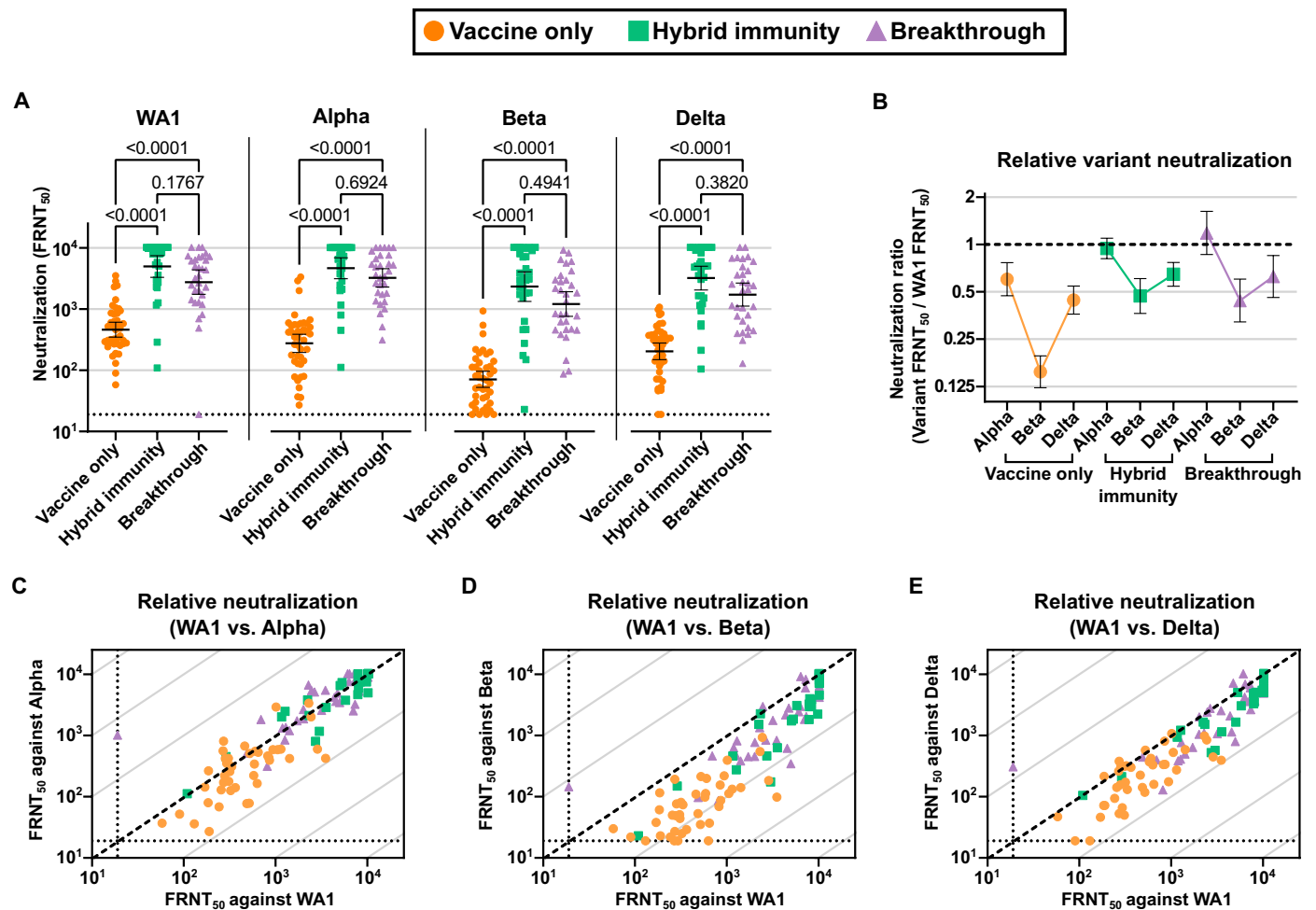
Fig. 2. Neutralizing antibody response after breakthrough infection, hybrid immunity, and vaccination alone. (**A**) Neutralizing antibody titers determined by focus-forming assay with clinical isolates of the original strain of SARS-CoV-2 (WA1), Alpha, Beta, and Delta variants. (**B**) The ratio of Alpha, Beta, and Delta variant neutralization to WA1 neutralization. WA1 neutralizing titer versus Alpha (**C**), Beta (**D**), and Delta (**E**) variant neutralizing titer. The dotted line indicates equal neutralization. Error bars in (A) and (B) indicate the geometric mean with the 95% confidence interval. *P* values in (A) were two-tailed and calculated with the Kruskal-Wallis method with Dunn's multiple comparison correction.

8 months after a second dose leads to improved overall Delta variant neutralizing titers by 6- to 12-fold (*23*). This appears consistent with the 8.5- and 15.7-fold improvements against the Delta variant for the breakthrough and hybrid immune groups, respectively, compared with two vaccine doses alone. This suggests that the magnitude of improvement for booster vaccinations may be similar to those seen with combined vaccination and natural infection, including hybrid immunity with a single dose of mRNA vaccine. This would point to the importance of the memory B cell compartment in generating a robust and variant cross-neutralizing humoral response. Although this study focuses on the humoral response, it is known that the cellular response by T cells plays an important role in responding to SARS-CoV-2 vaccination and infection (*24*).

COVID-19 vaccines using mRNA technology, including BNT162b2 and mRNA-1273, are the most commonly administered vaccines in the United States, where this study took place, and most of this study's participants received the BNT162b2 vaccine. However, some participants received the Ad26.COV2.S adenovirus-based vaccine. The majority of hybrid immunity research has focused on mRNA

vaccination, but research on adenovirus vaccine hybrid immunity has shown similar improvements to neutralizing titers and variant cross-neutralization (*25*). While this study was not designed to compare the effectiveness of different vaccination technologies, we do not anticipate any substantial effect due to differences in vaccine types.

Vaccination is highly effective at preventing the most severe outcomes from COVID-19 and should be provided regardless of previous infection status and age. A single dose of vaccine may provide sufficient protection for many individuals with previous SARS-CoV-2 infection. Vaccine availability remains limited in many regions, and the shortest path to broad global immunity may be to prioritize administering at least one vaccine dose to as many individuals as possible with a confirmed history of SARS-CoV-2 infection.

MATERIALS AND METHODS
Study design
The purpose of this study was to directly compare the humoral immune response among individuals who received COVID-19 vaccines either
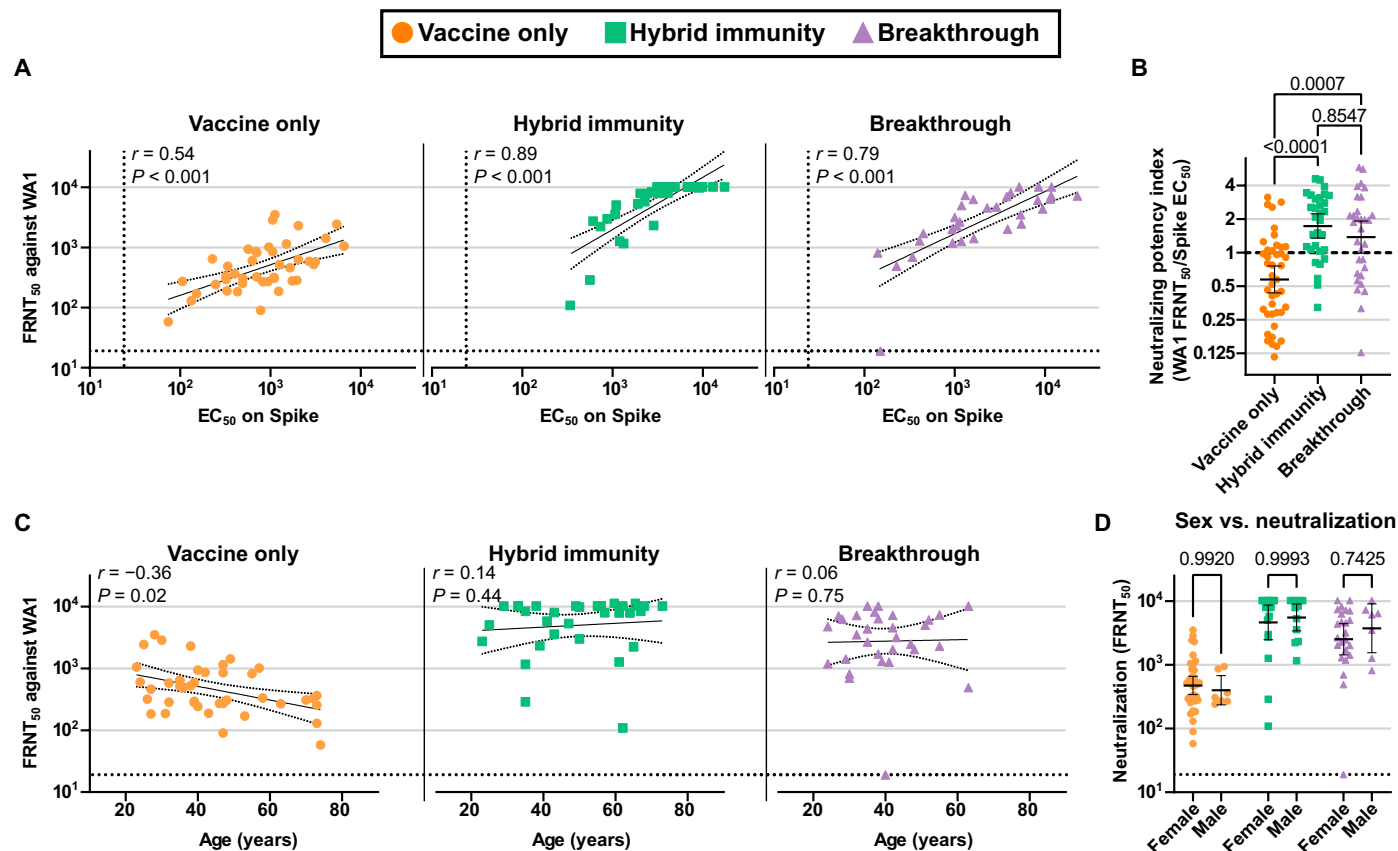
Fig. 3. Neutralizing efficiency and correlation with age. (**A**) Correlation between spike-specific EC_{50} values and WA1 neutralizing titers. (**B**) Serum-neutralizing potency index was calculated as the ratio of WA1 neutralizing titer to spike-specific EC_{50} values. (**C**) Correlation between age and WA1 neutralizing titers. (**D**) WA1 neutralization by sex. Error bars in (B) and (D) indicate the geometric mean with the 95% confidence interval. *P* values in (B) were two-tailed and calculated with the Kruskal-Wallis method with Dunn's multiple comparison correction. *P* values in (D) were two-tailed and calculated using a two-way analysis of variance (ANOVA) with the Šidák multiple comparison correction. Scatterplots in (A) depict the simple linear fit of log-transformed $FRNT_{50}$ versus log-transformed EC_{50} values with 95% confidence bands. Scatterplots in (C) depict the simple linear fit of log-transformed $FRNT_{50}$ versus age with 95% confidence bands. Correlations in (A) and (C) show Spearman's rank correlation coefficients and two-tailed *P* values.

before or after naturally acquired SARS-CoV-2 infection. Serum samples were collected from participants, which were analyzed using ELISAs, FRNTs, and measurement of ADCP. Study participants were selected for inclusion on the basis of a history of both vaccination and previous SARS-CoV-2 infection. Vaccinated controls with no history of previous infection were selected on the basis of sex, age, days between vaccine doses, and the time period since the most recent vaccination.

Cohort selection and serum collection

Health care workers at Oregon Health & Science University (OHSU) were recruited and enrolled in the study belonging to three groups: vaccine-only, hybrid immunity, and breakthrough infection. Written informed consent was obtained at the time of enrollment, and study approval was obtained from the OHSU institutional review board (IRB no. 00022511). Vaccine-only participants were fully vaccinated, defined as having received two doses of BNT162b2 or mRNA-1273, or one dose of Ad26.COV2.S. Serum samples were collected at least 14 days after the final vaccine dose. Hybrid immune participants had a history of PCR-confirmed diagnosis of COVID-19 at least 10 days before vaccination with at least one dose of

BNT162b2, mRNA-1273, or Ad26.COV2.S, and serum samples were collected at least 10 days after the final vaccine dose. Breakthrough participants were fully vaccinated as defined for the vaccine-only group at least 10 days before PCR-confirmed diagnosis of COVID-19, and serum samples were collected at least 10 days after the date of diagnosis. Sera were obtained by collecting 4 to 6 ml of whole blood in a BD Vacutainer Plus plastic serum tube, which was centrifuged at 1000*g* for 10 min before serum was aliquoted and stored at −20°C. Hybrid immune and breakthrough infection participants were selected on the basis of availability, whereas vaccine-only participants were selected to most closely match the average sex, age, and time since most recent vaccination (or infection for breakthrough) of the other two groups. Participants in these cohorts are previously described (*20*, *26*).

Enzyme-linked immunosorbent assays

ELISAs were performed as previously described (*20*). In 96-well plates (Corning Incorporated, EIA/RIA High Binding, reference no. 359096). Plates were coated with 100 μl per well of the following proteins at 1 μg/ml in phosphate-buffered saline (PBS) and incubated overnight at 4°C with rocking: SARS-CoV-2 RBD (produced in

Expi293F cells and purified using Ni-NTA chromatography), full-length SARS-CoV-2 spike (Recombinant Spike, SARS-CoV-2 stabilized protein, produced in Expi293F cells, BEI resources no. NR-52724), and nucleocapsid (SARS-CoV-2 Nucleocapsid-His, insect cell-expressed, SinoBio catalog no. 40588-V08B, item no. NR-53797, and lot no. MF14DE1611). Plates were washed three times with 0.05% (v/v) Tween 20 in PBS (wash buffer) and blocked with 150 µl per well and 5% nonfat dry milk powder in wash buffer (blocking buffer) at room temperature (RT) of about 20°C for 1 hour with rocking. Breakthrough and control sera were aliquoted and frozen in dilution plates and then resuspended in blocking buffer; sera were diluted and added to ELISA plates 100 µl per well (6 × 4-fold dilutions from 1:50 to 1:51,200), except for IgM (6 × 3-fold dilutions from 1:25 to 1:6075). Sera were incubated for 1 hour at RT before plates were filled three times with wash buffer. Secondary antibodies were added to plates at 100 µl per well depending on the intended readout: goat anti-human IgG/IgA/IgM–horseradish peroxidase (HRP) at 1:10,000 (Invitrogen, reference no. A18847), anti-human IgA-HRP at 1:3000 (BioLegend, reference no. 411002), mouse anti-human IgG-HRP clone G18-145 at 1:3000 (BD Biosciences, reference no. 555788), and goat anti-human IgM-HRP at 1:3000 (Bethyl Laboratories, reference no. A80-100P). Plates were incubated, protected from light with secondary antibody at RT for 1 hour with rocking, and then filled three times with wash buffer before the development with o-phenylenediamine dihydrochloride (Thermo Fisher Scientific, no. 34005) according to the manufacturer's 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. Normalized A_{492} values were calculated by subtracting the average of negative control wells and dividing by the 99th percentile of all wells from the same experiment. A dilution series of positive control serum was included on each plate to verify appropriate performance of the assay.

Cell culture
Vero E6 monkey kidney epithelial cells (CRL-1586) were obtained from the American Type Culture Collection (ATCC) and maintained in tissue culture-treated vessels in Dulbecco's modified Eagle's medium, 10% fetal bovine serum (FBS), 1% nonessential amino acids (NEAAs), and 1% penicillin-streptomycin (PS) (complete media) under tissue culture conditions (TCCs) of 100% relative humidity, 37°C, and 5% CO_2. THP-1 (ATCC, TIB-202) human monocyte cells were obtained from ATCC and maintained in suspension culture in tissue culture–treated vessels in Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% FBS, 1% NEAA, and 1% PS (THP-1 media).

SARS-CoV-2 growth and titration
SARS-CoV-2 isolates USA-WA1/2020 [lineage A] (NR-52281), USA/CA_CDC_5574/2020 [lineage B.1.1.7—alpha] (NR-54011), hCoV-19/South Africa/KRISP-K005325/2020 [lineage B.1.351—beta] (NR-54009), and hCoV-19/USA/PHC658/2021 [lineage B.1.617.2—delta] (NR-55611) were obtained from BEI Resources. Viral stocks were propagated as previously described (5). Subconfluent Vero E6 cells were infected at a multiplicity of infection of 0.05 in a minimal volume (0.01 ml/cm²) of Opti-MEM + 2% FBS (dilution media) for 1 hour at TCC, and then additional complete media (0.1 ml/cm²) was added and incubated for 24 hours at TCC. Culture supernatant was centrifuged for 10 min at 1000g and frozen at −80°C in aliquots.

Titration was performed on clear 96-well tissue culture plates containing 70 to 90% confluent (at the time of infection) Vero E6 cells. Dilutions (8 × 10-fold) were prepared in dilution media, and 30 µl per well of diluted virus was incubated with the cells for 1 hour at TCC before further addition of Opti-MEM, 2% FBS, and 1% methylcellulose (overlay media) and incubation for 24 hours at TCC. Plates were then fixed by soaking in 4% formaldehyde in PBS for 1 hour and then removing from the biosafety level three facility following institutional biosafety protocols. Cells were permeabilized in 0.1% bovine serum albumin and 0.1% saponin in PBS (perm buffer) for 30 min and then with polyclonal anti–SARS-CoV-2 alpaca serum (Capralogics Inc.) (1:5000 in perm buffer) overnight at 4°C. Plates were washed three times with 0.01% Tween 20 in PBS (focus wash buffer) and then incubated for 2 hours at RT with 1:20,000 anti-alpaca-HRP (Novus, no. NB7242). Plates were filled three times with focus wash buffer and then incubated with TrueBlue (SeraCare, no. 5510-0030) for 30 min or until sufficiently developed for imaging. Well 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 from the dilution factor and volume used during infection.

Focus reduction neutralization test
FRNT assays were carried out as previously described (5). Duplicate 5 × 4.7-fold (1:10 to 1:4879) serial dilutions of participant sera were prepared in 96-well plates. An equal volume of dilution media containing about 50 FFU of SARS-CoV-2 or variant was added to each well (final dilutions of sera, 1:20 to 1:9760) and incubated for 1 hour at TCC. Virus-serum mixtures were used to infect Vero E6 cells in 96-well plates as described above in the titration assay. Each plate contained 16 virus-only control wells, one for each serum dilution series. Fixation, development, and counting of FRNT plates were carried out as described above in the titration assay. Percent neutralization values were calculated for each well as the focus count divided by the average focus count of virus-only control wells from the same plate.

Antibody-dependent cellular phagocytosis
ADCP assay was adapted from a protocol described previously (28). Biotinylated RBD was incubated at 1 µg/ml with fluorescent neutravidin beads (Invitrogen, F8775) for 2 hours at RT; beads were washed twice with 1% bovine serum albumin in PBS (dilution buffer) and resuspended at a final dilution of 1:100 in dilution buffer. In a 96-well plate, 10 µl of resuspended bead solution was incubated with 10 µl of diluted serum from study participants for 2 hours at 37°C. After serum pretreatment, $2 × 10^4$ THP-1 cells were added to each well in 80 µl of THP-1 media and incubated overnight in TCC. The following morning, 100 µl of 4% paraformaldehyde was added to each well and incubated at least 30 min at RT before analysis on a CytoFLEX flow cytometer (Beckman Coulter). Samples were mixed for 3 s before analysis, and samples were injected until at least 2500 cell events were recorded per sample. Phagocytosis scores are reported as the product of percent bead-positive cells and mean fluorescence intensity of bead-positive cells and then divided by 10^6 for presentation. Three replicate experiments were performed for each participant serum sample, the average of which was used for further analysis. The gating strategy with representative data is presented in fig. S4.

Statistical analysis
$FRNT_{50}$ and EC_{50} values were calculated by fitting percent neutralization or normalized A_{492} values to a dose-response curve as previously

described (*5*). Final FRNT$_{50}$ values below the limit of detection (1:20) were set to 1:19. Final EC$_{50}$ values below the limit of detection of 1:25 for N, Spike, RBD, IgG, and IgA were set to 1:24, and values below 1:12.5 for IgM were set to 1:12. Aggregated EC$_{50}$ and FRNT$_{50}$ values were analyzed and plotted in GraphPad Prism (9.2.0). Dot plots of EC$_{50}$ and FRNT$_{50}$ values were generated on a log-transformed axis with error bars showing the geometric mean and 95% confidence interval. Phagocytosis score and neutralization ratio were plotted on a linear axis with error bars showing the arithmetic mean and 95% confidence interval. *P* values for dot plots were two-tailed and calculated using the Kruskal-Wallis test with Dunn's multiple comparison correction. *P* values for reported sex versus neutralization were two-tailed and calculated by group using a two-way ANOVA with the Šidák multiple comparison correction. Scatterplots were prepared by first log-transforming FRNT$_{50}$ and EC$_{50}$ data and then performing simple linear fitting and plotting the 95% confidence bands. Correlations were calculated using Spearman's correlation, and two-tailed *P* values were calculated for the 95% confidence interval.

SUPPLEMENTARY MATERIALS
www.science.org/doi/10.1126/sciimmunol.abn8014
Figs. S1 to S4
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES
1. E. Dong, H. Du, L. Gardner, An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect. Dis.* **20**, 533–534 (2020).
2. F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E. D. Moreira, C. Zerbini, R. Bailey, K. A. Swanson, S. Roychoudhury, K. Koury, P. Li, W. V. Kalina, D. Cooper, R. W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K. U. Jansen, W. C. Gruber; C4591001 Clinical Trial Group, Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
3. L. R. Baden, H. M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Rouphael, C. B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, E. D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B. S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* (2020).
4. S. Elbe, G. Buckland-Merrett, Data, disease and diplomacy: GISAID's innovative contribution to global health. *Glob. Challenges* **1**, 33–46 (2017).
5. T. A. Bates, H. C. Leier, Z. L. Lyski, S. K. McBride, F. J. Coulter, J. B. Weinstein, J. R. Goodman, Z. Lu, S. A. R. Siegel, P. Sullivan, M. Strnad, A. E. Brunton, D. X. Lee, A. C. Adey, B. N. Bimber, B. J. O'Roak, M. E. Curlin, W. B. Messer, F. G. Tafesse, Neutralization of SARS-CoV-2 variants by convalescent and BNT162b2 vaccinated serum. *Nat. Commun.* **12**, 5135 (2021).
6. R. E. Chen, X. Zhang, J. B. Case, E. S. Winkler, Y. Liu, L. A. VanBlargan, J. Liu, J. M. Errico, X. Xie, N. Suryadevara, P. Gilchuk, S. J. Zost, S. Tahan, L. Droit, J. S. Turner, W. Kim, A. J. Schmitz, M. Thapa, D. Wang, A. C. M. Boon, R. M. Presti, J. A. O'Halloran, A. H. J. Kim, P. Deepak, D. Pinto, D. H. Fremont, J. E. Crowe, D. Corti, H. W. Virgin, A. H. Ellebedy, P.-Y. Shi, M. S. Diamond, Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nat. Med.* **27**, 717–726 (2021).
7. J. Lopez Bernal, N. Andrews, C. Gower, E. Gallagher, R. Simmons, S. Thelwall, J. Stowe, E. Tessier, N. Groves, G. Dabrera, R. Myers, C. N. J. Campbell, G. Amirthalingam, M. Edmunds, M. Zambon, K. E. Brown, S. Hopkins, M. Chand, M. Ramsay, Effectiveness of Covid-19 vaccines against the B.1.617.2 (Delta) variant. *N. Engl. J. Med.* **385**, 585–594 (2021).
8. E. G. Levin, Y. Lustig, C. Cohen, R. Fluss, V. Indenbaum, S. Amit, R. Doolman, K. Asraf, E. Mendelson, A. Ziv, C. Rubin, L. Freedman, Y. Kreiss, G. Regev-Yochay, Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N. Engl. J. Med.* **385**, e84 (2021).
9. S. J. Thomas, E. D. Moreira Jr., N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. P. Marc, F. P. Polack, C. Zerbini, R. Bailey, K. A. Swanson, X. Xu, S. Roychoudhury, K. Koury, S. Bouguermouh, W. V. Kalina, D. Cooper, R. W. Frenck Jr., L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, Q. Yang, P. Liberator, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, W. C. Gruber, K. U. Jansen; C4591001 Clinical Trial Group, Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. *N. Engl. J. Med.* **385**, 1761–1773 (2021).
10. Y. Goldberg, M. Mandel, Y. M. Bar-On, O. Bodenheimer, L. Freedman, S. Alroy-Preis, N. Ash, A. Huppert, R. Milo, Protection and waning of natural and hybrid COVID-19 immunity. medRxiv 2021.12.04.21267114 (2021); www.medrxiv.org/content/10.1101/2021.12.04.21267114v1.
11. S. Nanduri, T. Pilishvili, G. Derado, M. M. Soe, P. Dollard, H. Wu, Q. Li, S. Bagchi, H. Dubendris, R. Link-Gelles, J. A. Jernigan, D. Budnitz, J. Bell, A. Benin, N. Shang, J. R. Edwards, J. R. Verani, S. J. Schrag, Effectiveness of Pfizer-BioNTech and Moderna vaccines in preventing SARS-CoV-2 infection among nursing home residents before and during widespread circulation of the SARS-CoV-2 B.1.617.2 (Delta) variant—National Healthcare Safety Network, March 1–August 1, 2021. *Morb. Mortal. Wkly. Rep.* **70**, 1163–1166 (2021).
12. S. Y. Tartof, J. M. Slezak, H. Fischer, V. Hong, B. K. Ackerson, O. N. Ranasinghe, T. B. Frankland, O. A. Ogun, J. M. Zamparo, S. Gray, S. R. Valluri, K. Pan, F. J. Angulo, L. Jodar, J. M. McLaughlin, Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: A retrospective cohort study. *Lancet* **398**, 1407–1416 (2021).
13. Y. M. Bar-On, Y. Goldberg, M. Mandel, O. Bodenheimer, L. Freedman, N. Kalkstein, B. Mizrahi, S. Alroy-Preis, N. Ash, R. Milo, A. Huppert, Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N. Engl. J. Med.* **385**, 1393–1400 (2021).
14. Z. Wang, F. Muecksch, D. Schaefer-Babajew, S. Finkin, C. Viant, C. Gaebler, H.-H. Hoffmann, C. O. Barnes, M. Cipolla, V. Ramos, T. Y. Oliveira, A. Cho, F. Schmidt, J. Da Silva, E. Bednarski, L. Aguado, J. Yee, M. Daga, M. Turroja, K. G. Millard, M. Jankovic, A. Gazumyan, Z. Zhao, C. M. Rice, P. D. Bieniasz, T. Hatziioannou, M. C. Nussenzweig, Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* **595**, 426–431 (2021).
15. J. S. Turner, J. A. O'Halloran, E. Kalaidina, W. Kim, A. J. Schmitz, J. Q. Zhou, T. Lei, M. Thapa, R. E. Chen, J. B. Case, F. Amanat, A. M. Rauseo, A. Haile, X. Xie, M. K. Klebert, T. Suessen, W. D. Middleton, P.-Y. Shi, F. Krammer, S. A. Teefey, M. S. Diamond, R. M. Presti, A. H. Ellebedy, SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature* **596**, 109–113 (2021).
16. M. Bergwerk, T. Gonen, Y. Lustig, S. Amit, M. Lipsitch, C. Cohen, M. Mandelboim, E. Gal Levin, C. Rubin, V. Indenbaum, I. Tal, M. Zavitan, N. Zuckerman, A. Bar-Chaim, Y. Kreiss, G. Regev-Yochay, Covid-19 breakthrough infections in vaccinated health care workers. *N. Engl. J. Med.* **385**, 1474–1484 (2021).
17. A. M. Cavanaugh, K. B. Spicer, D. Thoroughman, C. Glick, K. Winter, Reduced risk of reinfection with SARS-CoV-2 After COVID-19 vaccination—Kentucky, May–June 2021. *Morb. Mortal. Wkly. Rep.* **70**, 1081–1083 (2021).
18. W. F. Garcia-Beltran, E. C. Lam, M. G. Astudillo, D. Yang, T. E. Miller, J. Feldman, B. M. Hauser, T. M. Caradonna, K. L. Clayton, A. D. Nitido, M. R. Murali, G. Alter, R. C. Charles, A. Dighe, J. A. Branda, J. K. Lennerz, D. Lingwood, A. G. Schmidt, A. J. Iafrate, A. B. Balazs, COVID-19-neutralizing antibodies predict disease severity and survival. *Cell* **184**, 476–488.e11 (2021).
19. A. A. Butt, P. Yan, O. S. Shaikh, F. B. Mayr, Outcomes among patients with breakthrough SARS-CoV-2 infection after vaccination in a high-risk national population. *EClinicalMedicine* **40**, 101117 (2021).
20. T. A. Bates, H. C. Leier, Z. L. Lyski, J. R. Goodman, M. E. Curlin, W. B. Messer, F. G. Tafesse, Age-dependent neutralization of SARS-CoV-2 and P.1 variant by vaccine immune serum samples. *JAMA* **326**, 868–869 (2021).
21. H. S. Yang, V. Costa, S. E. Racine-Brzostek, K. P. Acker, J. Yee, Z. Chen, M. Karbaschi, R. Zuk, S. Rand, A. Sukhu, P. J. Klasse, M. M. Cushing, A. Chadburn, Z. Zhao, Association of age with SARS-CoV-2 antibody response. *JAMA Netw. Open* **4**, e214302 (2021).
22. Z. L. Lyski, A. E. Brunton, M. I. Strnad, P. E. Sullivan, S. A. R. Siegel, F. G. Tafesse, M. K. Slifka, W. B. Messer, SARS-CoV-2 specific memory B-cells from individuals with diverse disease severities recognize SARS-CoV-2 variants of concern. *J. Infect. Dis.* jiab585 (2021).
23. A. R. Falsey, R. W. Frenck, E. E. Walsh, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, R. Bailey, K. A. Swanson, X. Xu, K. Koury, W. Kalina, D. Cooper, J. Zou, X. Xie, H. Xia, Ö. Türeci, E. Lagkadinou, K. R. Tompkins, P.-Y. Shi, K. U. Jansen, U. Şahin, P. R. Dormitzer, W. C. Gruber, SARS-CoV-2 neutralization with BNT162b2 vaccine dose 3. *N. Engl. J. Med.* **385**, 1627–1629 (2021).
24. C. R. Moderbacher, S. I. Ramirez, J. M. Dan, A. Grifoni, K. M. Hastie, D. Weiskopf, S. Belanger, R. K. Abbott, C. Kim, J. Choi, Y. Kato, E. G. Crotty, C. Kim, S. A. Rawlings, J. Mateus, L. P. V. Tse, A. Frazier, R. Baric, B. Peters, J. Greenbaum, E. O. Saphire, D. M. Smith, A. Sette, S. Crotty, Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell* **183**, 996–1012.e19 (2020).
25. R. Keeton, S. I. Richardson, T. Moyo-Gwete, T. Hermanus, M. B. Tincho, N. Benede, N. P. Manamela, R. Baguma, Z. Makhado, A. Ngomti, T. Motlou, M. Mennen, L. Chinhoyi, S. Skelem, H. Maboreke, D. Doolabh, A. Iranzadeh, A. D. Otter, T. Brooks, M. Noursadeghi, J. C. Moon, A. Grifoni, D. Weiskopf, A. Sette, J. Blackburn, N.-Y. Hsiao, C. Williamson, C. Riou, A. Goga, N. Garrett, L.-G. Bekker, G. Gray, N. A. B. Ntusi, J. L. Moore, W. A. Burgers, Prior infection with SARS-CoV-2 boosts and broadens Ad26.COV2.S immunogenicity in a variant-dependent manner. *Cell Host Microbe.* **29**, 1611–1619.e5 (2021).

26. H. C. Leier, T. A. Bates, Z. L. Lyski, S. K. McBride, D. X. Lee, F. J. Coulter, J. R. Goodman, Z. Lu, M. E. Curlin, W. B. Messer, F. G. Tafesse, Previously infected vaccinees broadly neutralize SARS-CoV-2 variants. *medRxiv*, 2021.04.25.21256049 (2021).

27. L. C. Katzelnick, A. C. Escoto, B. D. McElvany, C. Chávez, H. Salje, W. Luo, I. Rodriguez-Barraquer, R. Jarman, A. P. Durbin, S. A. Diehl, D. J. Smith, S. S. Whitehead, D. A. T. Cummings, Viridot: An automated virus plaque (immunofocus) counter for the measurement of serological neutralizing responses with application to dengue virus. *PLOS Negl. Trop. Dis.* **12**, e0006862 (2018).

28. M. E. Ackerman, B. Moldt, R. T. Wyatt, A.-S. Dugast, E. McAndrew, S. Tsoukas, S. Jost, C. T. Berger, G. Sciaranghella, Q. Liu, D. J. Irvine, D. R. Burton, G. Alter, A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J. Immunol. Methods* **366**, 8–19 (2011).

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Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants

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