CORRESPONDENCE

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SARS-CoV-2 Antibody Response in Persons with Past Natural Infection

TO THE EDITOR: Whether or not persons who have already been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) should be vaccinated is unclear. Only a few studies have shown that vaccinees who were previously infected with SARS-CoV-2 had a significantly higher antibody response than previously uninfected vaccinees.[1-4] In an observational cohort study, we enrolled 100 health care workers, including 38 (9 men and 29 women) with a documented history of SARS-CoV-2 infection (mean duration between infection and vaccination, 111 days). The mean age of these previously infected participants was 35.1 years (95% confidence interval [CI], 31.7 to 38.6). Our study also included 62 participants (25 men and 37 women) who had not been previously infected. The mean age of those participants was 44.7 years (95% CI, 41.0 to 47.6).

Both groups of participants received the messenger RNA vaccine BNT162b2 (Pfizer–BioNTech). Serum samples were obtained from the previously infected participants 10 days after the administration of the first dose and from the previously uninfected participants 10 days after the administration of the second dose. Thereafter, all the participants were screened for the presence of specific anti–SARS-CoV-2 spike IgG by means of a chemiluminescence microparticle immunoassay.

No significant difference in circulating anti-spike IgG antibody titers was observed between the samples from previously infected participants (mean level, 20,120 arbitrary units per milliliter; 95% CI, 16,400 to 23,800) and those from previously uninfected participants (mean level, 22,639 arbitrary units per milliliter; 95% CI, 19,400 to 25,900) (median levels are shown in Fig. 1A). Circulating anti-spike IgG antibodies were not detected in only one previously infected participant; that participant did not have an antibody response to natural infection with SARS-CoV-2.

The same serum samples were also analyzed for the presence of specific anti–SARS-CoV-2 neutralizing antibodies. We observed a difference in levels of neutralizing antibodies between samples from the previously infected participants (geometric mean titer, 569; 95% CI, 467 to 670) and those from the previously uninfected participants (geometric mean titer, 118; 95% CI, 85 to 152) (P<0.001) (median levels are shown in Fig. 1B). No substantial differences were noted between the titers from the previously infected and the previously uninfected participants according to age (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org) or sex (data not shown).

The previously infected participants were categorized into three groups according to the time that had elapsed from infection to vaccination: 1 to 2 months (8 participants), more than 2 months to 3 months (17 participants), and more than 3 months (12 participants). The previously infected patient in whom circulating anti-spike IgG antibodies were not detected was not included in this categorization. The circulating IgG mean titers differed between the group vaccinated at 1 to 2 months and the group vaccinated at more than 2 months to 3 months after natural infection (mean level, 15,837 arbitrary units per milliliter [95% CI, 11,265 to 20,410] vs. 21,450 arbitrary units per milliliter [95% CI, 15,377 to 27,523]) (median levels are shown in Fig. 1C); however, because the number of participants was limited, a real distinction cannot be made. No further significant difference was observed between the group of participants vaccinated at more than 2 months to 3 months and the group of those vaccinated more than 3 months after infection (mean level, 21,090 arbitrary units per milliliter [95% CI, 14,702 to 27,477]).

The differences among the three groups were more evident with respect to levels of neutralizing antibodies, with geometric mean titers rang-

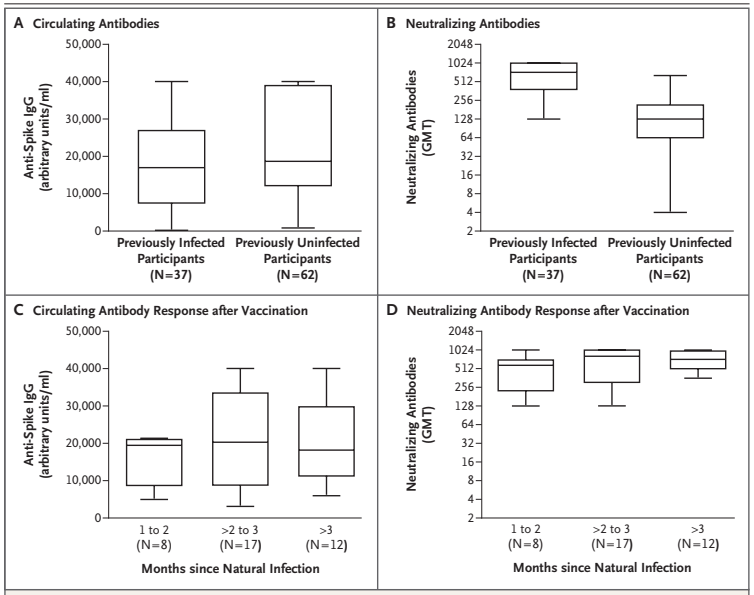

Figure 1. Immune Response in Participants with or without Previous SARS-CoV-2 Infection.

Shown are titers of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-spike IgG antibodies (Panel A) and neutralizing SARS-CoV-2 anti-spike IgG antibodies (Panel B) in serum samples obtained from previously infected participants after they received a single dose of vaccine and in samples obtained from previously uninfected participants after they received a second dose of vaccine. Differences in circulating (Panel C) and neutralizing (Panel D) IgG antibodies in samples obtained from previously infected participants were evaluated according to the duration from natural infection to vaccination (1 to 2 months, >2 months to 3 months, or >3 months). In each box-and-whisker plot, the horizontal line represents the median, the top and bottom of the box the interquartile range, and the whiskers the minimum and maximum values. GMT denotes geometric mean titer.

参考資料10

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ing from 437 (95% CI, 231 to 643) in participants vaccinated 1 to 2 months after infection to 559 (95% CI, 389 to 730) in those vaccinated more than 2 months to 3 months after infection to 694 (95% CI, 565 to 823) in those vaccinated more than 3 months after infection (median levels are shown in Fig. 1D). Although these findings indicate that the booster response was more efficacious when the vaccine was administered more than 3 months after infection, not enough information is available to draw a definitive conclusion.

The most remarkable finding of this study was the significantly lower neutralizing antibody titer after administration of a second dose of vaccine in previously uninfected patients than the titer after only a single dose of vaccine in previously infected participants. It is unclear how the neutralizing antibody titers influence the ability of the host to transmit the virus. These findings provide evidence that after the administration of a single dose of vaccine, the humoral response against SARS-CoV-2 in persons with a history of SARS-CoV-2 infection is greater than the response in previously uninfected participants who have received a second dose.

Gabriele Anichini, M.S.
Chiara Terrosi, M.S.
Claudia Gandolfo, Ph.D.
Gianni Gori Savellini, Ph.D.
University of Siena
Siena, Italy

Simonetta Fabrizi, M.D.
Giovanni B. Miceli, M.D.
Santa Maria alle Scotte University Hospital
Siena, Italy

M. Grazia Cusi, Ph.D.
University of Siena
Siena, Italy
mariagrazia.cusi@unisi.it

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Sotatercept for Pulmonary Arterial Hypertension

TO THE EDITOR: Humbert et al. (April 1 issue)[1] report that in the PULSAR trial, sotatercept reduced pulmonary vascular resistance in patients with pulmonary arterial hypertension by correcting dysregulated activin–growth differentiation factor signaling.[2] Sotatercept is also effective in increasing hemoglobin levels in patients with β-thalassemia.[3,4] The PULSAR trial excluded patients with hemoglobin levels above 16 g per deciliter at initial screening and above 18 g per deciliter after at least one dose of sotatercept. Depending on the prevalence of anemia and polycythemia among patients with pulmonary arterial hypertension, the erythropoietic effects of sotatercept could be consequential.

We performed a cross-sectional analysis involving a cohort of 366 patients referred because of dyspnea. On catheterization, these patients were categorized as having World Health Organization (WHO) group 1 pulmonary arterial hypertension, WHO group 2 to 5 pulmonary hypertension, or no pulmonary hypertension. Among the patients with group 1 pulmonary arterial hypertension, 49.4% had anemia (hemoglobin level, <12 g per deciliter in women and <13 g per deciliter in men). Patients with pulmonary arterial hypertension had lower hemoglobin levels, hematocrits, red-cell counts, and mean corpuscular hemoglobin concentrations and higher red-cell distribution widths than controls who did not have pulmonary arterial hypertension; these findings are similar to those in previous studies.[5]

In most patients with pulmonary arterial hypertension in our cohort (93.7%), the hemoglobin level was 16 g per deciliter or less. These patients would be expected to have a margin for the treatment-mediated increases of 1.2 to 1.5 g per

Neutralizing Response against Variants after SARS-CoV-2 Infection and One Dose of BNT162b2

TO THE EDITOR: The BNT162b2 vaccine was shown to have 95% efficacy against coronavirus disease 2019 (Covid-19).[1] To date, the two-dose vaccine protocol has not been approved in Israel for persons previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); however, administration of a single dose is now being considered.

In addition to the original virus first identified in Wuhan, China, SARS-CoV-2 variants first identified in the United Kingdom (B.1.1.7), South Africa (B.1.351), and Brazil (P.1) have been detected in recent months.[2] Samples from persons who had been vaccinated or previously infected with the original virus or the B.1.1.7 variant were shown to have significantly less neutralizing activity against the B.1.351 variant than against the other variants.[3,4] In this study, we investigated whether one dose of the BNT162b2 vaccine would increase neutralizing activity against the B.1.1.7, B.1.351, and P.1 variants in persons previously infected with SARS-CoV-2.

A microneutralization assay with isolates of the original virus (sublineage B.1) and the B.1.1.7,

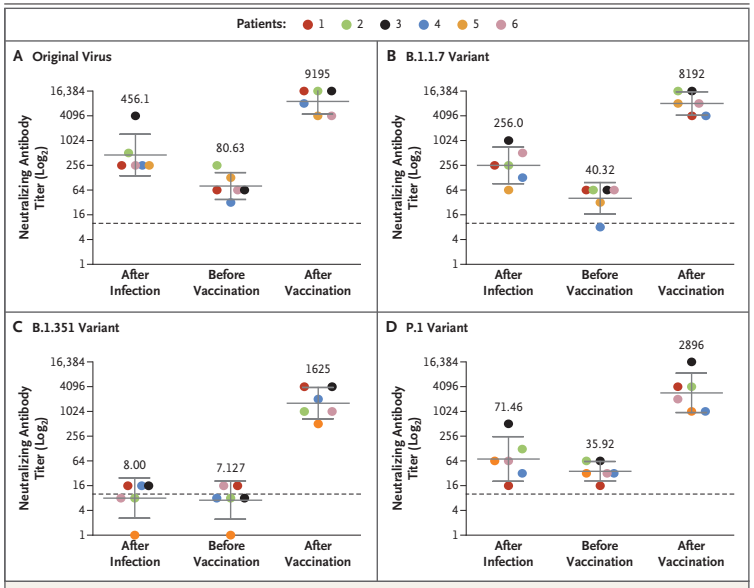

Figure 1. Neutralizing Response against the Original Virus and Variants after SARS-CoV-2 Infection and One Dose of the BNT162b2 Vaccine.

Serum samples from six patients previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), obtained 1 to 12 weeks after natural infection, immediately before receiving one dose of the BNT162b2 vaccine, and 1 to 2 weeks after vaccination, were tested with a microneutralization assay for the neutralizing response against sublineage B.1 of the original virus (Panel A), the B.1.1.7 variant first identified in the United Kingdom (Panel B), the B.1.351 variant first identified in South Africa (Panel C), and the P.1 variant first identified in Brazil (Panel D). Dashed lines indicate the cutoff titer. Solid lines and numbers indicate the geometric mean titer, and I bars show the 95% confidence interval.

B.1.351, and P.1 variants was performed on 18 serum samples from six health care workers previously infected with SARS-CoV-2, with a sample obtained from each patient at three time points: 1 to 12 weeks after natural infection, immediately before vaccination, and 1 to 2 weeks after vaccination (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). All six health care workers were women (32 to 67 years of age) and had been infected with the original virus (sublineage B.1), as determined by sequencing of SARS-CoV-2 performed at the time of diagnosis. Samples obtained at the first time point had neutralizing activity against the original virus and the B.1.1.7 and P.1 variants, with geometric mean titers of 456, 256, and 71, respectively, but had little or no neutralizing activity against the B.1.351 variant, with a geometric mean titer of 8. At the second time point, geometric mean titers were 81, 40, 36, and 7 for the original virus and the B.1.1.7, P.1, and B.1.351 variants, respectively. Of note, at the third time point, geometric mean titers were 9195, 8192, 2896, and 1625 for the original virus and the B.1.1.7, P.1, and B.1.351 variants, respectively — that is, the titers after vaccination were 114, 203, 81, and 228 times as high as the titers immediately before vaccination (Fig. 1 and Table S2).

This study showed that, in our small cohort, one vaccine dose substantially increased neutralizing activity against all variants tested, with similar titers detected across patients for each variant. This highlights the importance of vaccination even in previously infected patients, given the added benefit of an increased antibody response to the variants tested. Limitations of the study include the small cohort of only women and the lack of evaluation of T-cell response. However, we think the fact that all six patients responded similarly to vaccination supports our conclusions. Further studies could investigate the effects of a second vaccine dose on neutralizing activity against variants of concern in persons who have and persons who have not been previously infected.

Yaniv Lustig, Ph.D.
Ella Mendelson, Ph.D.
Michal Mandelboim, Ph.D.

Ministry of Health
Tel Hashomer, Israel
michalman@sheba.health.gov.il

and Others

Drs. Lustig and Nemet and Ms. Kliker contributed equally to this letter.

A complete list of authors is available with the full text of this letter at NEJM.org.

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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CORRECTION

STEP 1 for Effective Weight Control — Another First Step? (N Engl J Med 2021;384:1066-1067). The final sentence of the third paragraph (page 1067) should have ended, ". . . multiple endocrine neoplasia type 2," rather than ". . . type 1." In Table 1 (page 1067), under "GLP-1 Agonists," Dulaglutide should have been included, directly below "Weekly injection." The editorial is correct at NEJM.org.

This correction notice was updated on July 15, 2021, at NEJM.org.

INSTRUCTIONS FOR LETTERS TO THE EDITOR

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CORRESPONDENCE

Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine

TO THE EDITOR: The efficacy of two injections of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike messenger RNA (mRNA) vaccines (BNT162b2 [Pfizer] and mRNA-1273 [Moderna])[1] in preventing symptomatic SARS-CoV-2 infection in persons without previous coronavirus disease 2019 (Covid-19) has been shown to be high.[2,3] We wondered what the response would be to the first vaccine dose in persons with previous Covid-19.

We took advantage of our ongoing institutional review board–approved, longitudinal PARIS (Protection Associated with Rapid Immunity to SARS-CoV-2) study to provide a limited snapshot of the antibody responses in 110 study participants with or without documented preexisting SARS-CoV-2 immunity (mean age overall, 40.0 years [range, 24 to 68; ≥60 years, 8%]; 67 seronegative participants [64% female] with a mean age of 41.3 years and 43 seropositive participants [59% female] with a mean age of 41.4 years) (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org) who received their first spike mRNA vaccine dose in 2020 (88 received the Pfizer vaccine and 22 the Moderna vaccine). SARS-CoV-2 spike IgG was measured with the use of a previously described two-step enzyme-linked immunosorbent assay and expressed as area under the curve (AUC).[4,5]

Repeated sampling after the first dose indicates that the majority of seronegative participants had variable and relatively low SARS-CoV-2 IgG responses within 9 to 12 days after vaccination (median AUC before vaccination, 1 [67 participants]; at 0 to 4 days, 1 [12 participants]; at 5 to 8 days, 1 [22 participants]; at 9 to 12 days, 439 [13 participants]; at 13 to 16 days, 1016 [18 participants]; at 17 to 20 days, 1037 [21 participants]; at 21 to 27 days, 1293 [19 participants]; and after the second dose, 3316 [36 participants]) (Fig. 1A). In contrast, participants with SARS-CoV-2 antibodies at baseline before the first vaccine injection rapidly developed uniform, high antibody titers within days after vaccination (median AUC before vaccination, 90 [43 participants]; at 0 to 4 days, 133 [7 participants]; at 5 to 8 days, 14,208 [15 participants]; at 9 to 12 days, 20,783 [8 participants]; at 13 to 16 days, 25,927 [20 participants]; at 17 to 20 days, 11,755 [4 participants]; at 21 to 27 days, 19,534 [14 participants]; and after the second dose, 22,509 [19 participants]) (Fig. 1A).

The antibody titers of vaccinees with preexisting immunity were 10 to 45 times as high as those of vaccinees without preexisting immunity at the same time points after the first vaccine dose (e.g., 25 times as high at 13 to 16 days) and also exceeded the median antibody titers measured in participants without preexisting immunity after the second vaccine dose by more than a factor of 6. Although the antibody titers of the vaccinees without preexisting immunity increased by a factor of 3 after the second vaccine dose, no increase in antibody titers was observed in the Covid-19 survivors who received the second vac-

THIS WEEK'S LETTERS

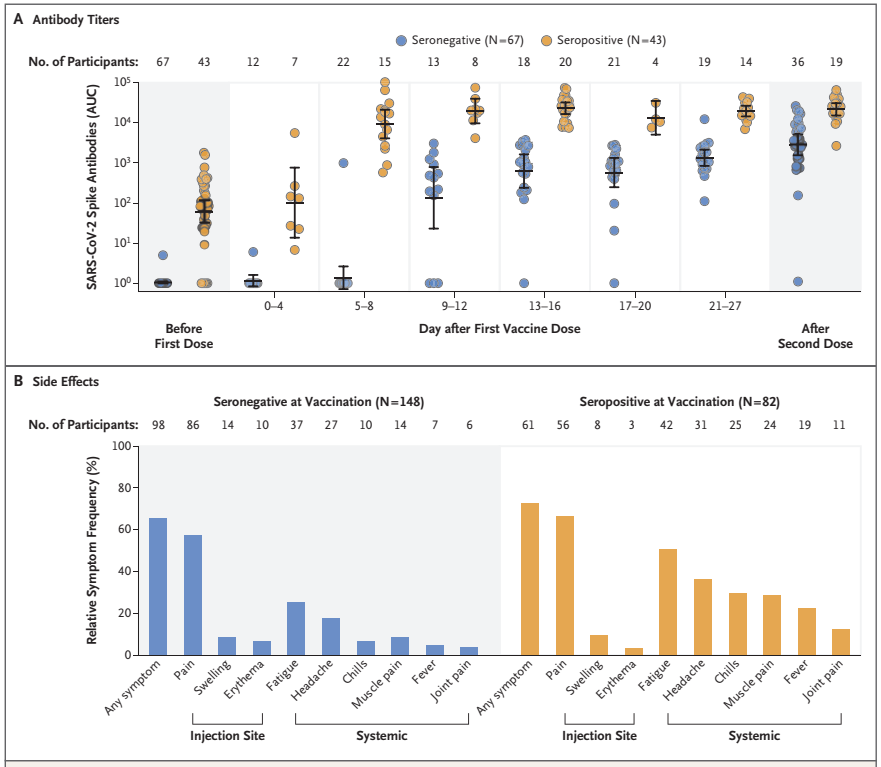

Figure 1. Immunogenicity and Reactogenicity of SARS-CoV-2 RNA Vaccines.

Panel A shows the quantitative SARS-CoV-2 spike antibody titers (assessed by means of enzyme-linked immunosorbent assay and expressed as area under the curve [AUC]) for 110 participants. Some participants with preexisting immunity had antibody titers below detection (AUC of 1) at the time point before vaccination. Geometric means with 95% confidence intervals (not adjusted for multiple testing) are shown. Panel B shows the relative frequency of vaccine-associated side effects after the first vaccine dose (230 participants). The local side effects occurred with similar frequency among participants with preexisting immunity and among those without preexisting immunity, whereas the systemic symptoms were more common among participants with preexisting immunity. The bars represent the relative frequency of each symptom, and the numbers at the top of the graph represent the absolute numbers for a given symptom, with a given participant possibly having more than one symptom.

cine dose. No substantial difference was noted in the dynamics of antibody responses elicited by the Pfizer and Moderna vaccines after the first dose (Fig. S1). The current analysis represents a convenience sample in which not all participants were able to provide biospecimens for antibody analysis at all the additional time intervals. Ongoing follow-up studies will show whether these early differences in immune responses are maintained over a prolonged time period.

In addition, we compared the frequency of local, injection-site–related as well as systemic reactions after the first dose of vaccine in 230 participants (mean age, 39.2 years [range, 22 to 70; ≥60 years, 8%]; 148 seronegative participants [70% female] and 82 seropositive participants [64% female]) (Fig. 1B). Overall, both vaccines (156 participants received the Pfizer vaccine and 74 the Moderna vaccine) had no side effects that resulted in hospitalization. A total of 159 of the 230 participants (69%) who completed the PARIS study survey reported having some side effects after the first vaccine dose (46% of the seronegative survey respondents and 89% of the seropositive survey respondents). Most common were localized injection-site symptoms (pain, swelling, and erythema), which occurred with equal frequency independently of the serostatus at the time of vaccination and resolved spontaneously within days after vaccination. Vaccine recipients with preexisting immunity had systemic side effects at higher frequencies than those without preexisting immunity (fatigue, headache, chills, muscle pain, fever, and joint pain, in order of decreasing frequency) (Fig. 1B). Because a convenience sample was used and only participants with available data were studied, caution is needed until the full data set, including side effects occurring after the first as well as the second vaccine dose, can be assessed.

We found that a single dose of mRNA vaccine elicited rapid immune responses in seropositive participants, with postvaccination antibody titers that were similar to or exceeded titers found in seronegative participants who received two vaccinations. Whether a single dose of mRNA vaccine provides effective protection in seropositive persons requires investigation.

Florian Krammer, Ph.D.
Komal Srivastava, M.S.
Hala Alshammary, M.S.
Angela A. Amoako, M.S.
Mahmoud H. Awawda, M.S.
Katherine F. Beach, B.S.
Maria C. Bermúdez-González, M.P.H.
Dominika A. Bielak, B.A.
Juan M. Carreño, Ph.D.
Rachel L. Chernet, B.A.
Lily Q. Eaker, B.A.
Emily D. Ferreri, B.S.
Daniel L. Floda, B.A.
Charles R. Gleason, B.S.
Joshua Z. Hamburger, M.D.
Kaijun Jiang, M.S.
Giulio Kleiner, Ph.D.
Denise Jurczyszak, Ph.D.
Julia C. Matthews, B.A.
Wanni A. Mendez, A.A.S.
Ismail Nabeel, M.D.
Lubbertus C.F. Mulder, Ph.D.
Ariel J. Raskin, B.A.
Kayla T. Russo, B.S.
Ashley-Beathrese T. Salimbangon, B.A.
Miti Saksena, M.B., B.S.
Amber S. Shin, B.S.
Gagandeep Singh, Ph.D.
Levy A. Sominsky, B.A.
Daniel Stadlbauer, Ph.D.
Ania Wajnberg, M.D.
Viviana Simon, M.D., Ph.D.
Icahn School of Medicine at Mount Sinai
New York, NY
florian.krammer@mssm.edu
viviana.simon@mssm.edu

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Sitagliptin for Prophylaxis of Acute Graft-versus-Host Disease

TO THE EDITOR: The trial reported by Farag et al. (Jan. 7 issue)[1] showed impressively low rates of severe acute graft-versus-host disease (GVHD) when sitagliptin was used in combination with tacrolimus and methotrexate after allogeneic stem-cell transplantation. However, these results

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BRIEF COMMUNICATION

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OPEN

Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2

Joseph E. Ebinger[1], Justyna Fert-Bober[1,2], Ignat Printsev[3], Min Wu[1], Nancy Sun[1], John C. Prostko[4], Edwin C. Frias[4], James L. Stewart[4], Jennifer E. Van Eyk[1,2], Jonathan G. Braun[3,5,6]✉, Susan Cheng[1,6]✉ and Kimia Sobhani[5,6]✉

In a cohort of BNT162b2 (Pfizer–BioNTech) mRNA vaccine recipients ($n=1,090$), we observed that spike-specific IgG antibody levels and ACE2 antibody binding inhibition responses elicited by a single vaccine dose in individuals with prior SARS-CoV-2 infection ($n=35$) were similar to those seen after two doses of vaccine in individuals without prior infection ($n=228$). Post-vaccine symptoms were more prominent for those with prior infection after the first dose, but symptomology was similar between groups after the second dose.

Messenger RNA (mRNA) vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19), offer great promise for curbing the spread of infection[1–3]. Challenges to the supply chain have prompted queries around whether single-dose administration rather than double-dose administration might suffice for certain individuals, including those recovered from prior infection. Emerging immune data, including detectable presence of anti-SARS-CoV-2 antibodies and virus-specific T cells, have suggested possible alternate vaccination strategies for previously infected individuals[4–6]. Recent small studies have indicated that individuals with prior infection might have naturally acquired immunity that could be sufficiently enhanced by a single dose rather than a double dose of administered vaccine[7,8]. To this end, we evaluated SARS-CoV-2-specific antibody responses after the first and second doses of the BNT162b2 (Pfizer–BioNTech) mRNA vaccine in a large and diverse cohort of healthcare workers. We compared the responses of individuals with confirmed prior infection to those of individuals without prior evidence of infection.

We enrolled healthcare workers from across a large academic medical center in Southern California. Vaccine recipients ($n=1,090$) who provided at least one blood sample for antibody testing were aged 41.9 ± 12.2 years and were 60.7% female and 53.3% non-White (Table 1): 981 vaccine recipients, including 78 with prior SARS-CoV-2 infection, provided baseline (pre-vaccine) samples; 525 (35 with prior infection) provided samples after dose 1; and 239 (11 with prior infection) provided samples after dose 2. A total of 217 individuals (ten with prior infection) provided blood samples at all three time points. Antibody levels were measured at three time points: before or up to 3 d after dose 1; within 7–21 d after dose 1; and within 7–21 d after dose 2. Because the timing of the first blood draw for antibody testing could confound the association of spike glycoprotein-specific IgG (IgG(S-receptor-binding domain (RBD))) with prior infection versus early vaccine response[9], we used nucleoprotein-specific IgG (IgG(N)) to denote prior SARS-CoV-2 exposure while recognizing a small potential for representing cross-reactivity with other coronaviruses. Given that the BNT162b2 vaccine delivers mRNA encoding only for spike protein, the expected elicited response is production of IgG(S-RBD) antibodies and not IgG(N) antibodies;[10] furthermore, IgG(N) antibodies are also known to represent a durable marker and indicator of post-infectious status[11]. Accordingly, we determined prior SARS-CoV-2 infection status and timing in relation to the first vaccine date, based on concordance of data documented in health records, presence of any IgG(N) antibodies at baseline pre-vaccination testing and the self-reported survey information collected. All cases of data discrepancy regarding prior SARS-CoV-2 infection status underwent manual physician adjudication, including medical chart review for evidence of positive SARS-CoV-2 polymerase chain reaction (PCR) or antibody testing that could have been performed by outside institutions or otherwise documented in the medical record.

For both IgG(N) (representing response to prior infection) and IgG(S-RBD) (representing response to either prior infection or vaccine), as expected, individuals with prior SARS-CoV-2 infection had higher antibody levels at all time points ($P\leq0.001$) (Supplementary Tables 1 and 2 and Extended Data Fig. 1). Notably, IgG(S-RBD) levels were only slightly lower in previously infected individuals at baseline when compared to infection-naive individuals who had received a single vaccine dose (log-median AU ml^{-1} (interquartile range, 6.0 (4.6, 6.9) versus 7.0 (6.3, 7.6)), $P<0.001$). Moreover, IgG(S-RBD) levels were not significantly different among previously infected individuals after a single dose and infection-naive individuals who had received two doses (10.0 (9.2, 10.4) versus 9.9 (9.4, 10.3), $P=0.92$) (Fig. 1). Similar results were found in a sensitivity analysis including only individuals who had antibody immunoassays performed at all three time points (Supplementary Tables 3 and 4). Specifically, those with prior infection had higher IgG(S-RBD) than

[1]Department of Cardiology, Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [2]Advanced Clinical Biosystems Institute, Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [3]F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [4]Applied Research and Technology, Abbott Diagnostics, Abbott Park, IL, USA. [5]Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [6]These authors contributed equally: Jonathan G. Braun, Susan Cheng, Kimia Sobhani. ✉e-mail: jonathan.braun2@cshs.org; susan.cheng@cshs.org; kimia.sobhani@cshs.org

Table 1 | Characteristics of the study cohort

	Total	Pre-vaccine	Post-vaccine dose 1	Post-vaccine dose 2
n	1,090	981	525	239
Age in years, mean (s.d.)	41.89 (12.18)	41.60 (12.05)	43.66 (12.79)	44.12 (12.65)
Race, *n* (%)				
White	509 (46.7)	453 (46.2)	263 (50.1)	130 (54.4)
Black or African American	36 (3.3)	33 (3.4)	22 (4.2)	9 (3.8)
Asian	300 (27.5)	265 (27.0)	154 (29.3)	67 (28.0)
Native Hawaiian/Pacific Islander	29 (2.7)	27 (2.8)	14 (2.7)	3 (1.3)
American Indian/Alaska Native	2 (0.2)	2 (0.2)	0 (0.0)	0 (0.0)
Multiple/other	139 (12.8)	130 (13.2)	58 (11.1)	27 (11.4)
Prefer not to answer	75 (6.9)	71 (7.2)	14 (2.6)	3 (1.3)
Ethnicity, *n* (%)				
Hispanic/Latinx	139 (12.8)	126 (12.8)	55 (10.5)	20 (8.4)
Non-Hispanic/Latinx	881 (80.8)	788 (80.3)	460 (87.6)	216 (90.4)
Prefer not to answer	70 (6.4)	67 (6.8)	10 (1.9)	3 (1.3)
Sex, *n* (%)				
Male	362 (33.2)	331 (33.7)	159 (30.3)	65 (27.2)
Female	662 (60.7)	587 (59.8)	353 (67.2)	168 (70.3)
Other	1 (0.1)	1 (0.1)	1 (0.2)	1 (0.4)
Prefer not to answer	65 (6.0)	62 (6.3)	12 (2.3)	5 (2.1)
Prior SARS-CoV-2 infection, *n* (%)	86 (7.9)	78 (8.0)	35 (6.7)	11 (4.6)
Antibody levels, mean (%)				
Architect IgG index (S/C) (IgG(N))	0.30 (0.86)	0.25 (0.84)	0.36 (0.90)	0.34 (0.82)
Architect IgM index (S/C)	0.99 (2.41)	0.26 (1.24)	2.11 (4.11)	3.38 (5.96)
Architect Quant IgG II (AU ml^{-1}) (IgG(S-RBD))	2,801.04 (6,159.27)	103.90 (693.89)	3,183.38 (7,299.73)	24,084.06 (16,367.63)

those without prior infection at all time points. No difference in IgG(S-RBD) levels was detected between those with prior infection after one dose of vaccine and those without prior infection after two doses (10.2 (8.4, 10.5) versus 9.9 (9.4, 10.3), $P=0.58$).

For surrogate measures of antibody neutralization, we examined IgG(S-RBD) levels at or above 4,160 AU ml^{-1} given that this threshold corresponds to a 0.95 probability of obtaining a plaque reduction neutralization test (PRNT) ID_{50} at a 1:250 dilution. Proportions of IgG(S-RBD) $\geq$ 4,160 AU ml^{-1} were similar between previously infected individuals at baseline compared to infection-naive individuals after a single dose ($P=1.00$). Notably, these proportions were lower in previously infected individuals after a single dose than in infection-naive individuals after two doses ($P<0.001$); there were no between-group differences after two doses (Supplementary Table 5 and Extended Data Fig. 2). We also used an angiotensin-converting enzyme 2 (ACE2) binding inhibition assay that correlates well with the SARS-CoV-2 PRNT methodology and exhibits a high correlation with the IgG(S-RBD) assay threshold ($r^2=0.95$). We found that ACE2 binding inhibition was significantly higher among previously infected individuals than infection-naive individuals after a single vaccine dose (94.3% versus 37.3%, $P<0.001$), with no between-group difference seen after the second dose (100.0% versus 97.8%, $P=1.00$). In time-shifted analyses, there was no difference in ACE2 binding between individuals with prior SARS-CoV-2 infection after a single dose and infection-naive individuals after two doses (94.3% versus 97.8%, $P=0.52$) (Supplementary Table 6 and Extended Data Fig. 3).

In parallel with antibody response analyses, we also examined post-vaccine symptomology (Supplementary Tables 7–11 and Extended Data Fig. 4). We observed that previously infected individuals experienced significant post-vaccine symptoms (that is, reactogenicity) more frequently than infection-naive individuals after dose 1 (36.8% versus 25.0%, $P=0.03$). However, there was no between-group difference in significant symptoms after dose 2 (51.3% versus 58.7%, $P=0.26$). In time-shifted analyses, infection-naive individuals had higher reactogenicity after dose 2 than previously infected individuals after their first dose (58.7% versus 36.8%, $P<0.001$). Fever and chills were more common among previously infected vaccine recipients after the first dose, whereas infection-naive individuals were more likely to experience headache, dizziness or lightheadedness after the second dose. In analyses of changes from dose 1 to dose 2, reactogenicity increased in frequency for infection-naive individuals (25.0% versus 58.7%, $P<0.001$) and less so in previously infected individuals (36.8% versus 51.3%, $P=0.08$).

Overall, we found that individuals previously infected with SARS-CoV-2 developed vaccine-induced antibody responses after a single dose of the BNT162b2 (Pfizer–BioNTech) mRNA vaccine that were similar to antibody responses seen after a two-dose vaccination course administered to infection-naive individuals. Our findings in a large and diverse cohort of healthcare workers expand from the results of smaller studies that have indicated higher levels of anti-S antibodies at baseline, and after a single mRNA vaccine dose, in previously infected individuals compared to those without prior infection[7,8,12,13]. In a total cohort of over 1,000 vaccine recipients, including several hundred with blood sampling after administered vaccine doses, we found that the anti-S antibody response after a single vaccine dose in previously infected individuals was

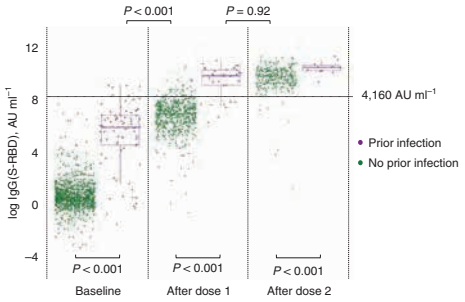

Fig. 1 | IgG(S-RBD) antibody response to mRNA SARS-CoV-2 vaccination in individuals with and without prior SARS-CoV-2 infection. Box plots display the median values with the interquartile range (lower and upper hinge) and ±1.5-fold the interquartile range from the first and third quartile (lower and upper whiskers). We used two-sided Wilcoxon tests, without adjustment for multiple testing, to perform the following between-group comparisons: (1) infection-naive individuals ($n = 903$) and those with prior infection ($n = 78$), both at baseline ($P < 0.001$); (2) infection-naive individuals (n = 490) and those with prior infection ($n = 35$), both after dose 1 ($P < 0.001$); (3) infection-naive individuals ($n = 228$) and those with prior infection ($n = 11$), both after dose 2 ($P < 0.001$); (4) infection-naive individuals ($n = 490$) after dose 1 and those with prior infection ($n = 78$), both at baseline ($P < 0.001$); and (5) infection-naive individuals ($n = 228$) after dose 2 and those with prior infection ($n = 35$) after dose 1 ($P = 0.92$).

similar to the response seen after two doses in all vaccine recipients irrespective of prior infection status. We further assessed the neutralization potential of elicited antibodies using a high-throughput ACE2 inhibition neutralization surrogate assay. Similarly to findings from a smaller study that directly measured antibody neutralizing capacity in 59 volunteers[8], we found, in our large cohort, that a second vaccine dose did not offer previously infected individuals a substantially greater benefit over a single dose in antibody neutralizing potential. Thus, our data suggest that a single dose of the Pfizer–BioNTech vaccine is sufficient for individuals with prior SARS-CoV-2 infection, not only when considering the response in anti-S antibody levels but also when examining results of an ACE2 inhibition assay indicating the potential neutralizing capability of elicited antibodies.

Limitations of our study include the 21-d timeframe within which antibodies were measured after each vaccine dose. Longer-term follow-up can provide additional information regarding the putative duration of immunity acquired from receiving a single dose versus a double dose of vaccine. Measures of T cell responses can shed further light on how a single dose versus a double dose of vaccine might be sufficient for augmenting T cell memory in previously infected individuals[12]. Further studies are needed to determine if a certain window for vaccination timing might be optimal to maximize efficacy as well as safety in previously infected individuals. Larger-sized cohorts are needed for sufficient statistical power to examine differences across demographic and clinical subgroups that are known to exhibit variation in antibody responses after vaccination[14–16]. When neutralizing capacity was estimated using a conservative IgG(S-RBD) threshold of >4,160 AU ml^{-1}, the single-dose response in previously infected individuals was numerically similar, albeit statistically significantly lower, than the antibody response after two doses in infection-naive individuals. When applying this conservative threshold of >4,160 AU ml^{-1}, which correlates with a 95% probability of high neutralizing antibody titer[17], statistical comparisons are susceptible to the influence of extreme values in the context of smaller-sized subgroups. Notably, there was no significant difference in the surrogate measure of ACE2 binding inhibition between persons with and without prior infection in time-shifted analysis after vaccine dose 1 and dose 2, respectively. Notwithstanding methodological differences between examining IgG(S-RBD) levels and assays of ACE2 binding inhibition, these surrogate measures suggest materially similar levels of achieved neutralization capacity. Some variation in antibody responses might also be related to heterogeneity within previously infected individuals, including in timing and severity of prior illness. Although circulating antibody levels alone are not definitive measures of immune status, serial measures of the serological response to either natural or inoculated exposures are known to correlate well with effective protective immunity[18], and our results indicate their potential utility in guiding vaccine deployment strategies for both previously infected and infection-naive individuals.

Our results offer preliminary evidence in support of a middle ground between public health-motivated and immunologically supported vaccine strategies. If validated, an approach that involves providing a single dose of vaccine to individuals with a confirmed history of SARS-CoV-2 infection along with an on-time complete vaccine schedule for infection-naive individuals could maximize the benefit of a limited vaccine supply.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01325-6.

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Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals

Rapid vaccine-induced population immunity is a key global strategy to control COVID-19. Vaccination programmes must maximise early impact, particularly with accelerated spread of new variants.[1] Most vaccine platforms use a two-dose prime-boost approach to generate an immune response against the virus S1 spike protein, the titres of which correlate with functional virus neutralisation and increase with boosting.[2,3] To enable larger numbers of people to receive the first dose, delayed administration of the second dose has been advocated and implemented by some.[1] The impact of previous SARS-CoV-2 infection on the need for boosting is not known.

We reasoned that previous infection could be analogous to immune priming. As such, a first prime vaccine dose would effectively act as boost, so a second dose might not be needed. To test this, we undertook a nested case-control analysis of 51 participants of COVIDsortium,[4,5] an ongoing longitudinal observational study of health-care workers (HCWs) in London who underwent weekly PCR and quantitative serology testing from the day of the first UK lockdown on March 23, 2020, and for 16 weeks onwards. 24 of 51 HCWs had a previous laboratory-confirmed mild or asymptomatic SARS-CoV-2 infection, as confirmed by positive detection of antibodies against the SARS-CoV-2 nucleocapsid (Elecsys Anti-SARS-CoV-2 N ECLIA, Roche Diagnostics, Burgess Hill, UK) or the receptor binding domain of the SARS-CoV-2 S1 subunit of the spike protein (anti-S; Elecsys anti-SARS-CoV-2 spike ECLIA, Roche Diagnostics), whereas 27 HCWs remained seronegative. A median of 12·5 sampling timepoints per participant permitted the identification of peak antibody titres in seropositive individuals while avoiding false negatives.

All participants received their first dose of the BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech, Mainz, Germany)[2,3] and were tested 19–29 days later (median 22 days, IQR 2). Among previously uninfected, seronegative individuals, anti-S titres after one vaccine dose were comparable to peak anti-S titres in individuals with a previous natural infection who had not yet been vaccinated. Among those with a previous SARS-CoV-2 infection, vaccination increased anti-S titres more than 140-fold from peak pre-vaccine levels (figure). This increase appears to be at least one order of magnitude greater than reported after a conventional prime-boost vaccine strategy in previously uninfected individuals.[3]

These serological data suggest that for individuals receiving the BNT162b2 mRNA vaccine, a potential approach is to include serology testing at or before the time of first vaccination to prioritise use of booster doses for individuals with no previous infection. This could potentially accelerate vaccine rollout. With increasing variants (UK, South Africa, Brazil), wider coverage without compromising vaccine-induced immunity could help reduce variant emergence. Furthermore, reactogenicity after unnecessary boost risks an avoidable and unwelcome increase in vaccine hesitancy.

Whether enhanced vaccine-induced antibody responses among previously seropositive individuals will show differential longevity compared to boosted vaccines remains to be seen. In the meantime, our findings provide a rationale for serology-based vaccine dosing to maximise coverage and impact.

CM and ADO contributed equally. AS and JCM contributed equally. All authors' contributions are listed in the appendix. Funding details for this Correspondence are provided in the appendix. DMA and RJB have consulted as members of Global T cell Expert Consortium, Oxford Immunotec, UK. All other authors declare no competing interests. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

*Charlotte Manisty, Ashley D Otter, Thomas A Treibel, Áine McKnight, Daniel M Altmann, Timothy Brooks, Mahdad Noursadeghi, *Rosemary J Boyton, Amanda Semper, James C Moon*
r.boyton@imperial.ac.uk

Institute of Cardiovascular Science (CM, TAT, JCM), Division of Infection and Immunity (MN), University College London, London, UK; Department of Cardiology, St Bartholomew's Hospital, Barts Health NHS Trust, London, UK (CM, TAT, JCM); National Infection Service, Public Health England, Porton Down, UK (ADO, TB, AS); The Blizard Institute, Queen Mary University of London School of Medicine and Dentistry, London, UK (AM); Department of Immunology and Inflammation (DMA); Department of Infectious Disease (RJB) Imperial College London, London W12 0NN, UK; Lung Division, Royal Brompton and Harefield Hospitals, London, UK (RJB)

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See **Online** for appendix

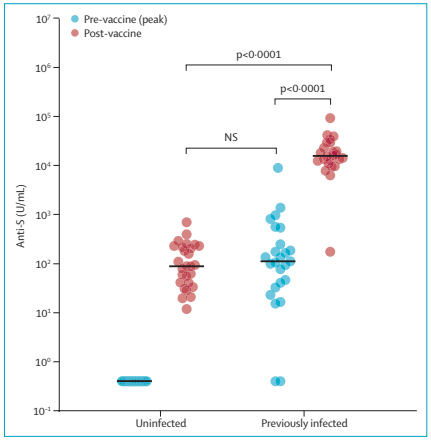

Figure: **Serological response to one dose of the BNT162b2 mRNA COVID-19 vaccine in individuals with and without laboratory-confirmed previous SARS-CoV-2 infection**
SARS-CoV-2 anti-S antibody titres in individuals with no previous infection are similar to titres in individuals who have had a mild SARS-CoV-2 infection. Anti-S titres in those with previous SARS-CoV-2 infection are more than 140-fold greater than at time of peak infection. Statistical analysis was by unpaired two-tailed *t* test. U=unit. NS=non-significant.

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Undoing supremacy in global health will require more than decolonisation

I read with interest Seye Abimbola and Madhukar Pai's Perspective.[1] It provides an enlightening and hopeful vision of decolonised global health detangled from supremacy in its many forms. However, it left me feeling that the vast mark that colonisation has left on society, politics, and system hierarchy within low-income and middle-income countries (LMICs) has been less considered. Without paying due consideration to the challenges of supremacy and oppression within LMICs, we cannot realistically equalise global health and progress to ensure that it upholds health equity and social justice.

Globally, we observe how rich academics in high-income countries (HICs), particularly from the UK and USA, tend to get richer. For example, the ways in which global health funding and publication are dominated by prominent academics and high-income prestigious institutions mean that worthy work can be dismissed when teams are less valued. Importantly, many individuals from LMICs who are valuable in directing global health endeavours do not have the opportunities or training to prove why or how they are valuable in meaningful ways to academia. Under some circumstances, they can be actively oppressed.

There is a refusal to learn from local populations, especially those from the margins of society, and ethnic superiority exists within societal, political, and academic structures in both HICs and LMICs, which is rising amid right-wing conservatism in some settings. How do we effectively empower valuable leaders to push forward necessary global health measures when they are restricted from the outset?

Colonisation has left a pervasive mark. Its legacy in LMICs still needs to be unpicked. Creating truly equitable global health must involve diverse groups of people who view challenges through differing lenses from their backgrounds, lived experiences, and skills, and who have wider, inclusive visions that do not focus on individual career success and are not at the mercy of prescribed academic agendas in HICs.

I declare no competing interests.

Keerti Gedela
keertigedela@gmail.com

Chelsea and Westminster NHS Foundation Trust, London SW10 9NH, UK

1 Abimbola S, Pai M. Will global health survive its decolonisation? *Lancet* 2020; **396:** 1627–28.

Seye Abimbola and Madhukar Pai[1] describe eloquently how, for historical reasons, global health is operationalised as a saviourism model. To redress the balance of power between saviour and saved, they envision a utopic global health fuelled by respect and humility, and motivated by an adherence to values based on rights, equity, and justice.

Unfortunately, the disciplines that dominate global health attend to the causes of and solutions to disease endpoints on the health and wellbeing spectrum. Such disciplines have not engaged adequately with a crucial understanding of the sociostructural production of health or with the political arguments based on myriad values that fall outside of the traditional medical and health sciences. It is impossible to decolonise global health if crucial geopolitical analyses, and the impact on relationships between high-income countries (HICs) and low-income and middle-income countries (LMICs), remain chronically marginalised.

Additionally, decolonising global health extends beyond relations between LMICs and HICs; it is also about the relationships within them. Decolonisation is fundamentally about redressing inequity and power imbalance. It cannot be achieved without also addressing gender inequity, racism, and other forms of structural violence. The colonised also have to be at least as reflective about the status quo as the colonisers. This mindset goes beyond engagement and participation between HICs and LMICs, to disrupting the norms of dependency within LMICs that enable the inequities and replicate the hierarchies of neocolonialism. In real terms, LMICs must confront their own internal power relations inherent in the discourse of immutable culture, which protect cronyism, tribalism, poor governance, and patriarchy.

Ultimately, a decolonised global health can only exist within a broader geopolitical and economic environment that supports rights, equity, and justice.

We declare no competing interests.

**Pascale Allotey, Daniel D Reidpath*
pascale.allotey@unu.edu

International Institute for Global Health, United Nations University, Bandar Tun Razak, Kuala Lumpur 56000, Malaysia (PA); International Centre for Diarrhoeal Disease Research Bangladesh, Dhaka, Bangladesh (DDR)

1 Abimbola S, Pai M. Will global health survive its decolonisation? *Lancet* 2020; **396:** 1627–28.

Authors' reply

We thank Keerti Gedela as well as Pascale Allotey and Daniel Reidpath for their responses to our Perspective on decolonising global health.[1] We welcome and completely agree with the points they highlighted for additional emphasis: greater

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CORONAVIRUS

Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals after mRNA vaccination

Rishi R. Goel[1,2†], Sokratis A. Apostolidis[1,2,3†], Mark M. Painter[1,2†], Divij Mathew[1,2†], Ajinkya Pattekar[2], Oliva Kuthuru[1], Sigrid Gouma[4], Philip Hicks[4], Wenzhao Meng[1,5], Aaron M. Rosenfeld[1,5], Sarah Dysinger[4], Kendall A. Lundgreen[4], Leticia Kuri-Cervantes[1,4], Sharon Adamski[2], Amanda Hicks[2], Scott Korte[2], Derek A. Oldridge[1,5], Amy E. Baxter[1], Josephine R. Giles[1,6,7], Madison E. Weirick[4], Christopher M. McAllister[4], Jeanette Dougherty[1], Sherea Long[1], Kurt D'Andrea[1], Jacob T. Hamilton[2,4], Michael R. Betts[1,4], Eline T. Luning Prak[1,5], Paul Bates[4], Scott E. Hensley[4], Allison R. Greenplate[1,2], E. John Wherry[1,2,6,7]*

mRNA vaccines for SARS-CoV-2 have been authorized for emergency use. Despite their efficacy in clinical trials, data on mRNA vaccine–induced immune responses are mostly limited to serological analyses. Here, we interrogated antibody and antigen-specific memory B cells over time in 33 SARS-CoV-2 naïve and 11 SARS-CoV-2 recovered subjects. SARS-CoV-2 naïve individuals required both vaccine doses for optimal increases in antibodies, particularly for neutralizing titers against the B.1.351 variant. Memory B cells specific for full-length spike protein and the spike receptor binding domain (RBD) were also efficiently primed by mRNA vaccination and detectable in all SARS-CoV-2 naïve subjects after the second vaccine dose, although the memory B cell response declined slightly with age. In SARS-CoV-2 recovered individuals, antibody and memory B cell responses were significantly boosted after the first vaccine dose; however, there was no increase in circulating antibodies, neutralizing titers, or antigen-specific memory B cells after the second dose. This robust boosting after the first vaccine dose strongly correlated with levels of preexisting memory B cells in recovered individuals, identifying a key role for memory B cells in mounting recall responses to SARS-CoV-2 antigens. Together, our data demonstrated robust serological and cellular priming by mRNA vaccines and revealed distinct responses based on prior SARS-CoV-2 exposure, whereby COVID-19 recovered subjects may only require a single vaccine dose to achieve peak antibody and memory B cell responses. These findings also highlight the utility of defining cellular responses in addition to serologies and may inform SARS-CoV-2 vaccine distribution in a resource-limited setting.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has resulted in hundreds of millions of infections and millions of deaths worldwide (*1*). Novel vaccines have recently been issued emergency use authorization by the U.S. Food and Drug Administration and are being widely administered (*2*, *3*). Early data from clinical trials suggest that these vaccines are safe and effective (*4*, *5*); however, there is still a paucity of information on how these novel mRNA vaccines elicit immune responses at the cellular and molecular level.

The humoral immune response to infection or vaccination results in two major outcomes: the production of antibodies by antibody-secreting cells (ASCs) that can provide rapid serological immunity and the generation of long-lived memory B cells capable of mounting recall responses (*6*, *7*). If circulating antibodies fail to confer protection to a future exposure, memory B cells drive the recall response by producing new antibodies through forming new ASCs or reentering germinal centers for additional rounds of somatic hypermutation (SHM) (*8*, *9*). In the context of acute severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, immunological memory in the form of antibodies and memory B cells are durable for over 8 months after symptom onset (*10*–*14*). However, studies on vaccinated individuals have largely focused on measuring binding and/or neutralizing antibodies as primary end points (*15*–*17*), and the induction of memory B cells by mRNA vaccines remains poorly understood. Although antibodies are a central component of vaccine efficacy, memory B cells may be important for long-term protection, responses to subsequent infection, and the ability to respond to emerging variant strains (*18*). Furthermore, it is unclear how memory B cell responses relate to serological responses for novel SARS-CoV-2 mRNA vaccines, and how memory B cell responses differ after vaccination in subjects who previously experienced SARS-CoV-2 infection compared with those who are SARS-CoV-2 naïve.

A related question is whether individuals who experienced prior SARS-CoV-2 infection require a second dose of mRNA vaccine. As these individuals have already generated a primary immune response to SARS-CoV-2 during their natural infection, it is possible that a single dose of vaccine could be sufficient to boost antibody and memory B cell responses. This question is particularly relevant in settings of limited vaccine supply and challenging vaccine deployment (*19*). Several recent studies have indicated that antibody responses can be robustly induced in SARS-CoV-2 experienced individuals, consistent with an anamnestic response (*20*–*23*). Although one study suggests that memory B cells might also be boosted after a single vaccine dose (*24*), it remains unclear how memory B cell responses are affected by the second dose of mRNA vaccine in SARS-CoV-2 naïve versus recovered individuals. These key gaps in our understanding require longitudinal analysis of antibodies together with memory B cell responses after the first and second dose of mRNA vaccine in SARS-CoV-2 naïve and experienced subjects.

Here, we established a longitudinal cohort of SARS-CoV-2 naïve and SARS-CoV-2 recovered individuals who received SARS-CoV-2 mRNA vaccines. From these longitudinal samples, we assessed both circulating antibodies and antigen-specific memory B cells over the course of first and second immunization. We also compared vaccine responses with demographic and clinical metadata, including age and side effects. These data offer new insights into the B cell response to SARS-CoV-2 mRNA vaccines.

RESULTS

For this study, we recruited 44 healthy individuals (i.e., no self-reported chronic health conditions) who received SARS-CoV-2 mRNA vaccines (Pfizer BNT162b2 or Moderna mRNA-1273) at the University of Pennsylvania Health System. Full cohort information is described in fig. S1. Of this cohort, 11 individuals had a prior SARS-CoV-2 infection, ranging from 65 to 275 days before vaccination. Peripheral blood samples were collected for immunological analysis at four key time points (Fig. 1A): prevaccine baseline (time point 1), 2 weeks after the first dose (time point 2), the day of second dose (time point 3), and 1 week after the second dose (time point 4). This study design allowed us to investigate the kinetics of immune responses after both primary and secondary immunizations.

Antibody responses to SARS-CoV-2 mRNA vaccination

We first measured circulating antibody responses in longitudinal serum samples by enzyme-linked immunosorbent assay (ELISA). At baseline, SARS-CoV-2 naïve individuals had undetectable levels of immunoglobulin G (IgG) antibodies specific for either full-length spike protein or the spike receptor binding domain (RBD) (Fig. 1B). Primary vaccination induced a significant increase in SARS-CoV-2–specific antibodies, which was further enhanced by the booster dose (Fig. 1B). In contrast, all SARS-CoV-2 recovered individuals had detectable levels of anti-spike and anti-RBD IgG at baseline, and these antibody responses were significantly increased after the first dose of vaccine (Fig. 1B). However, in SARS-CoV-2 recovered subjects, there was no additional increase in antibody levels after the second vaccine dose (Fig. 1B). Notably, the levels of anti-RBD IgG were similar in the SARS-CoV-2 naïve and SARS-CoV-2 recovered individuals at 1 week after boost (time point 4) (Fig. 1B).

In addition to total spike- and RBD-binding antibody, we further assessed antibody function using a pseudovirus neutralization assay. Specifically, we tested the ability of vaccine-induced sera to neutralize pseudotyped virus expressing either the D614G (the initial dominant strain at the time of the study) spike protein or the B.1.351 variant (originally referred to as the South African variant; now called Beta) spike protein. SARS-CoV-2 naïve individuals had a moderate response to primary immunization, with ~50% of participants developing detectable levels of neutralizing antibodies against D614G 2 weeks after primary (Fig. 1, C and D). In contrast, primary immunization was largely ineffective to induce functional antibodies against the B.1.351 variant with only 4 of 25 individuals developing neutralizing titers above limit of detection (LOD) over the same time frame (Fig. 1, C and D). Neutralizing titers were significantly increased after the second dose in SARS-CoV-2 naïve individuals, with all participants achieving neutralization against D614G and 26 of 27 achieving detectable neutralization against B.1.351 at 7 days after boost (Fig. 1, C and D). Consistent with anti-spike and anti-RBD antibody levels, SARS-CoV-2 experienced individuals had a robust increase in neutralizing antibodies after primary immunization, with no further increase in neutralization titers against D614G and B.1.351 after the second dose (Fig. 1C). The first dose of vaccine also appeared to resolve baseline differences in neutralization between D614G and B.1.351 in this group (Fig. 1D).

On the basis of these data, we quantified the relationship between total antibody levels and neutralization ability in SARS-CoV-2 naïve individuals to assess the relative quality of antibodies induced by the first and second dose of mRNA vaccine. Before the second dose, anti-spike antibodies were only moderately correlated with neutralizing titers against D614G, with further drop-off for the B.1.351 variant (Fig. 1E). Preboost anti-RBD antibodies were more predictive of neutralization titers against D614G and B.1.351 (Fig. 1E) than anti-spike antibodies. Both anti-spike and anti-RBD antibodies correlated more strongly with neutralizing titers against D614G and B.1.351 after the second dose (Fig. 1E), indicating a marked improvement in the quality of the antibody response. Together, these data supported the importance of a two-dose regimen for effective antibody responses, especially against the B.1.351 variant, in SARS-CoV-2 naïve individuals. Conversely, a single dose of vaccine was able to achieve highly effective antibody responses in SARS-CoV-2 recovered individuals with no further improvement postboost.

Memory B cell responses to SARS-CoV-2 mRNA vaccination

We next asked how mRNA vaccination affected the responses of memory B cells specific for SARS-CoV-2. To address this question, we developed a flow cytometric assay using a combination of fluorescently labeled antigens as probes to track the induction of virus-specific memory B cells in longitudinal peripheral blood mononuclear cell (PBMC) samples (fig. S2A) (*11*, *13*, *25*). Analysis of bulk B cell populations revealed no change in the frequency of naïve, non-naïve, or memory B cells across the time course of vaccination, or between SARS-CoV-2 naïve and recovered individuals (fig. S2B), highlighting the stability of the overall B cell compartment.

Despite a stable frequency of total memory B cells, there were marked changes in SARS-CoV-2 antigen–specific B cell populations in response to vaccination. Consistent with the antibody data, SARS-CoV-2 naïve individuals had minimal spike-specific memory B cells at baseline, whereas SARS-CoV-2 recovered individuals had a significant population of spike-specific memory B cells ranging from ~0.15 to 0.8% of total memory B cells (Fig. 2, A and B). Memory B cells targeting the spike RBD followed a similar trend and the frequency of these antigen-specific memory B cells was comparable to a separate cohort of nonvaccinated SARS-CoV-2 recovered donors (Fig. 2, A and B). After primary immunization, SARS-CoV-2 naïve individuals had a significant increase in spike-specific and RBD-specific memory B cells over baseline (Fig. 2B). These memory B cells were also significantly boosted after administration of the second vaccine

[1]Institute for Immunology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [2]Immune Health, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [3]Division of Rheumatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [4]Department of Microbiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [5]Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [6]Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA. [7]Parker Institute for Cancer Immunotherapy, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.
*Corresponding author. Email: wherry@pennmedicine.upenn.edu
†These authors contributed equally to this work.

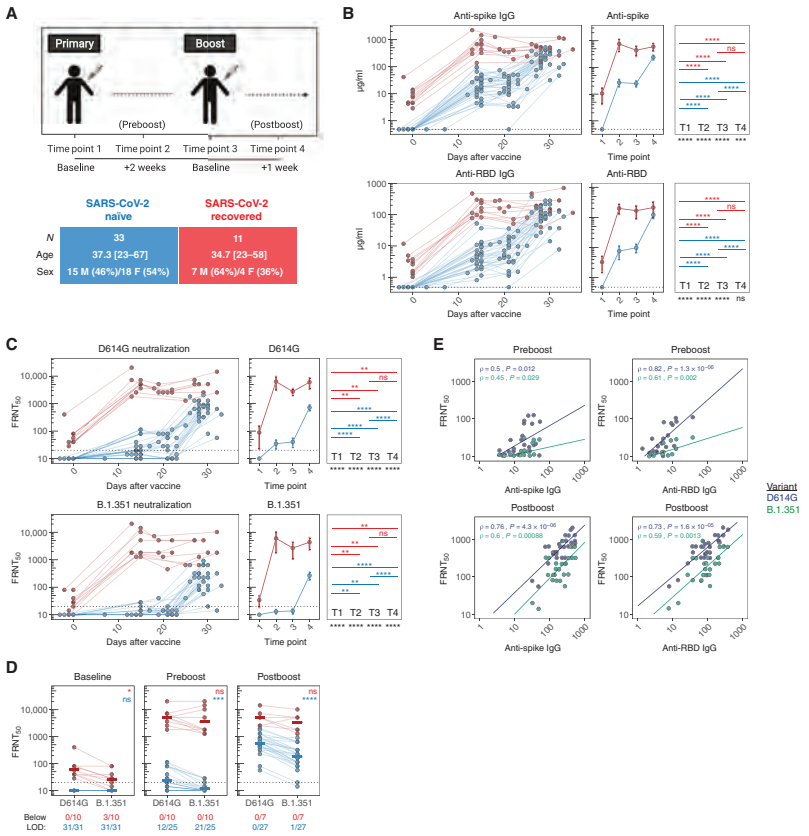

Fig. 1. Antibody responses after mRNA vaccination in SARS-CoV-2 naïve and recovered individuals. (**A**) University of Pennsylvania Immune Health COVID vaccine study design. (**B**) Concentration of anti-spike and anti-RBD IgG antibodies in vaccinated individuals over time. (**C**) $FRNT_{50}$ of vaccine-induced sera against pseudotyped virus expressing SARS-CoV-2 D614G (wild-type) or B.1.351 (South African) variant spike protein. (**D**) Paired analysis of neutralization titers against D614G and B.1.351 in vaccine-induced sera at baseline (time point 1), preboost (time point 2), and postboost (time point 4). (**E**) Bivariate analysis of total anti-spike and anti-RBD binding antibodies with pseudovirus neutralization titers against D614G and B.1.351. Associations between total antibody levels and neutralizing ability were calculated using Spearman's rank correlation and are shown with linear trend lines. Dotted lines indicate the LOD for the assay. Statistics were calculated using unpaired Wilcoxon test (comparisons between time points and comparisons between naïve and recovered) or paired Wilcoxon test (comparisons between D614G and B.1.351) with Holm correction for multiple comparisons. Blue and red values indicate statistical comparisons within naïve or recovered groups. Black values indicate statistical comparisons between naïve and recovered groups.

dose, approaching the levels of memory B cells observed in nonvaccinated SARS-CoV-2 recovered donors (Fig. 2B). In contrast, SARS-CoV-2 recovered individuals had a robust expansion of spike- and RBD-specific memory B cells after primary immunization but had no additional boosting after the second vaccine dose (Fig. 2B). As a control, we also examined the frequency of influenza hemagglutinin (HA)–specific memory B cells in both SARS-CoV-2 naïve and recovered individuals after SARS-CoV-2 vaccination. The frequency of these antigen-unrelated memory B cells remained stable throughout the mRNA vaccination time course (Fig. 2B), confirming the specificity of this memory B cell assay. Together, these results demonstrated robust induction of SARS-CoV-2–specific memory B cells by two doses of mRNA vaccine in SARS-CoV-2 naïve subjects. In contrast, a single dose of mRNA vaccine amplified

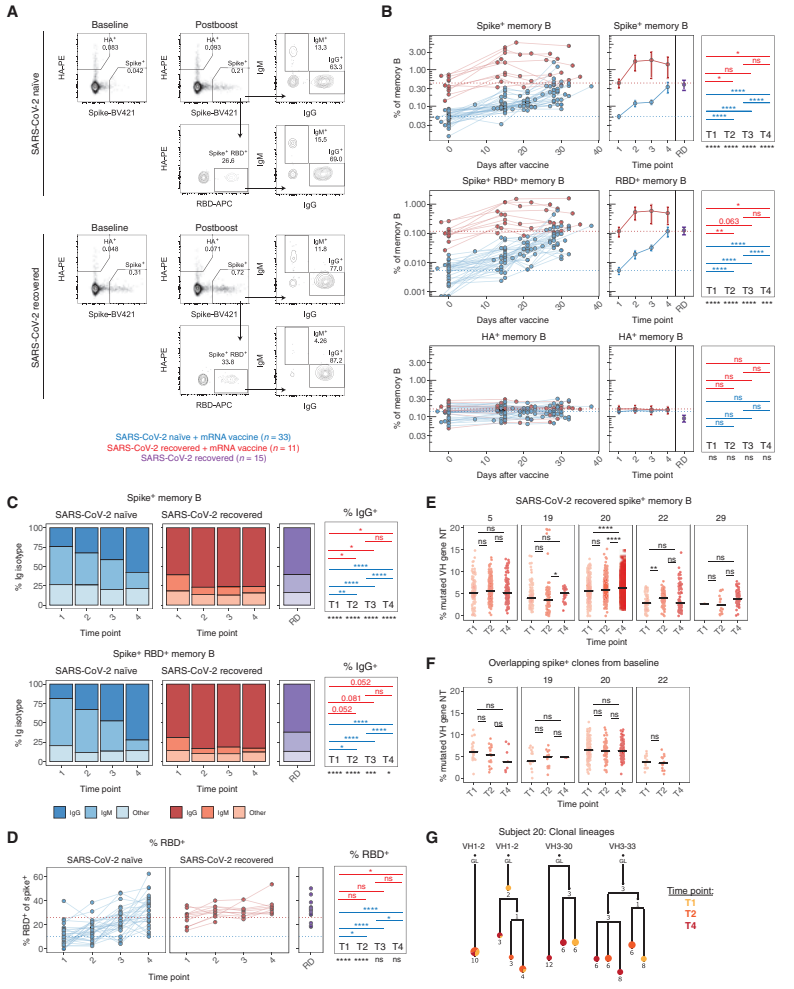

Fig. 2. Antigen-specific memory B cell responses after mRNA vaccination in SARS-CoV-2 naïve and recovered individuals. (**A**) Gating strategy and representative plots for flow cytometric analysis of SARS-CoV-2–specific B cells. (**B**) Frequency of spike$^+$, spike$^+$/RBD$^+$, and HA$^+$ memory B cells over time in vaccinated individuals. Data are represented as frequency of antigen-specific cells in the total memory B cell compartment. (**C**) Frequency of IgG and IgM isotypes over time in the antigen-specific memory B cell compartments. (**D**) Frequency of RBD$^+$ memory B cells over time in vaccinated individuals, as a percentage of spike$^+$ memory B cells. (**E**) SHM status of spike$^+$ memory B cell clones over time in SARS-CoV-2 recovered individuals. Data are represented as percent of VH gene nucleotides that are mutated. (**F**) SHM of productive spike-binding clones sampled at time point 1, which were also found in at least one other time point. Clones with fewer than 10 copies in each patient were excluded. (**G**) Clonal evolution of spike-binding memory B cell lineages that were present before vaccination in a recovered individual. For representative lineages, numbers refer to mutations compared with the preceding vertical node. Colors indicate time point, black dots indicate inferred nodes, and size is proportional to sequence copy number; GL, germline sequence. All panels: Dotted lines indicate the mean at baseline. RD, nonvaccinated, SARS-CoV-2 recovered donors. Statistics were calculated using unpaired Wilcoxon test (comparisons between time points and comparisons between naïve and recovered) with Holm correction for multiple comparisons. Blue and red values indicate statistical comparisons within naïve or recovered groups. Black values indicate statistical comparisons between naïve or recovered groups.

preexisting antigen-specific memory B cells in SARS-CoV-2 recovered subjects, with no additional quantitative benefit after the second vaccine dose.

We further analyzed the phenotype of SARS-CoV-2–specific memory B cells. On day 15 after primary immunization, ~25 to 30% of spike-specific memory B cells were IgG^+ and ~40 to 50% were IgM^+ in SARS-CoV-2 naïve individuals (Fig. 2C). The frequency of IgG^+ memory B cells increased to >50% after the second dose of vaccine in these subjects (Fig. 2C), consistent with a qualitative improvement in memory B cells after the boost. Conversely, in SARS-CoV-2 recovered individuals, ~60 to 70% of spike-specific memory B cells detected before vaccination were IgG^+ (Fig. 2C). Although the frequency of IgG^+ memory B cells increased slightly to ~75% after the first dose of vaccine, further increases were not observed after the second immunization (Fig. 2, C and D). A similar pattern of IgG frequency was observed for RBD-specific memory B cells (Fig. 2C). In addition, the fraction of spike-specific memory B cells that recognized RBD remained stable over time in SARS-CoV-2 recovered individuals. In SARS-CoV-2 naïve subjects, the fraction of the overall spike-specific memory B cell response that was focused on RBD increased over time, becoming equivalent to that observed in SARS-CoV-2 recovered individuals after the second vaccine dose (Fig. 2D). Overall, these data indicated a qualitative benefit to the virus-specific memory B cell response after both doses of vaccine in SARS-CoV-2 naïve individuals and qualitative improvement after the first but not the second vaccine dose in SARS-CoV-2 recovered subjects.

Last, we sorted $spike^+$ memory B cells from five recovered donors at baseline (time point 1), postprimary (time point 2), and postboost (time point 4) for B cell receptor (BCR) sequencing to further evaluate potential changes in the memory B cell response induced by vaccination. SHM is a process of DNA point hypermutation that occurs in immunoglobulin variable gene sequences and usually accompanies T cell–dependent B cell responses within germinal centers (*26*). Accordingly, SHM is a frequently used marker for the evaluation of immune memory (*27*). Here, SHM was calculated as the average percentage of mutated VH gene nucleotides in each clone, counting each clone only once. Full sequencing information, including the number of clones identified for each sample, is listed in table S3. Mutational analysis of total spike-binding memory clones revealed a modest shift toward higher SHM at the postprimary and postboost time point in some individuals (Fig. 2E); however, there was no clear pattern across the five individuals measured. To determine whether SHM changed within preexisting spike-binding clones, we next looked for high-copy spike-binding clones that were shared between the baseline time point and at least one other time point. These clones, which were present before the first vaccine dose, presumably arose during the initial infection with SARS-CoV-2. Subject 29 was not included in this analysis because there was only one clone that met the copy number cutoff. SHM levels in the overlapping clones did not increase after vaccination (Fig. 2F). The stability of SHM could also be seen within lineage trees for subject 20, who had the largest number of clones sampled. Specifically, the nodes (sequence variants) within lineages exhibited mixing between the time points, and where they were separate, they were not consistently found at higher frequencies in parts of the trees with higher levels of SHM (Fig. 2G and fig. S3). These data suggested that preexisting spike-specific memory clones in SARS-CoV-2 recovered individuals did not increase their level of SHM in response to either dose of vaccine.

Demographic and clinical factors associate with B cell responses to SARS-CoV-2 mRNA vaccination

In addition to prior SARS-CoV-2 exposure, we also investigated associations between other demographic and clinical metadata with vaccine-induced B cell responses. Several previous studies have reported a negative association between age and vaccine-induced antibody titers after a single dose of mRNA vaccines (*28*, *29*). We therefore investigated potential relationships between sex or age and B cell responses after one or two doses of vaccine. In our cohort of SARS-CoV-2 naïve vaccinees, there were no associations between sex and antibody or memory B cell responses (Fig. 3, A and B). While there was no association between age and anti-spike IgG after the first immunization (i.e., preboost), there was a trend toward a negative relationship between age and preboost RBD-specific IgG (Fig. 3C). Antibody for both spike and RBD had a similarly negative, but statistically insignificant, correlation with age after the second vaccine dose (Fig. 3C). However, there was a clear negative correlation between the postboost frequency of antigen-specific memory B cells and age (Fig. 3D). Although this relationship represented weaker induction of memory B cells with older age, all age groups still displayed an increase in the frequency of SARS-CoV-2–specific memory B cells compared with prevaccine baseline (Fig. 3, C and D). There was also no change in the frequency of total memory B cells by sex or age, indicating the antigen-specific nature of this effect (fig. S4). Although our cohort is not extensively enriched in those over 50 years old and does not directly address elderly vaccinees, these data pointed to potentially relevant age-related changes in immune response to vaccination.

An additional question is whether vaccine-induced side effects have any relationship to immune responses (*20*). We addressed this question by comparing vaccine-induced antibody and memory B cell responses in subjects with or without self-reported systemic side effects (i.e., fever, chills, headache, fatigue, and myalgia; fig. S1C). In SARS-CoV-2 naïve vaccinees with systemic side effects after the second dose, there was a trend toward an increase in antibody responses at the postboost time point (Fig. 3E). Such a trend was not observed for the memory B cell response (Fig. 3E). We further investigated the potential association between reactogenicity and increased antibody response using a multivariate regression to control for the effects of sex and age. This multivariate analysis similarly revealed a positive association of systemic side effects with anti-spike and anti-RBD antibody levels 7 days after the booster immunization (Fig. 3F). Although these data only represent a statistical trend ($P = 0.051$), they do provoke questions about potential relationships between early vaccine-induced inflammation and the induction of antibody responses that should be addressed in future studies.

Relationships between antibody and memory B cell responses to SARS-CoV-2 mRNA vaccination

Last, we investigated the potential relationships between antibody and memory B cell responses. To address this question, we first performed hierarchical clustering of vaccine-induced B cell responses in SARS-CoV-2 naïve subjects. As expected, postboost (time point 4) samples clustered away from the earlier time points, with some subgrouping of patients based on the relative magnitude of antibody and memory B cell responses (Fig. 4A). Hierarchical clustering of the different readouts of antigen-specific humoral immunity also revealed that antibodies and memory B cells clustered separately

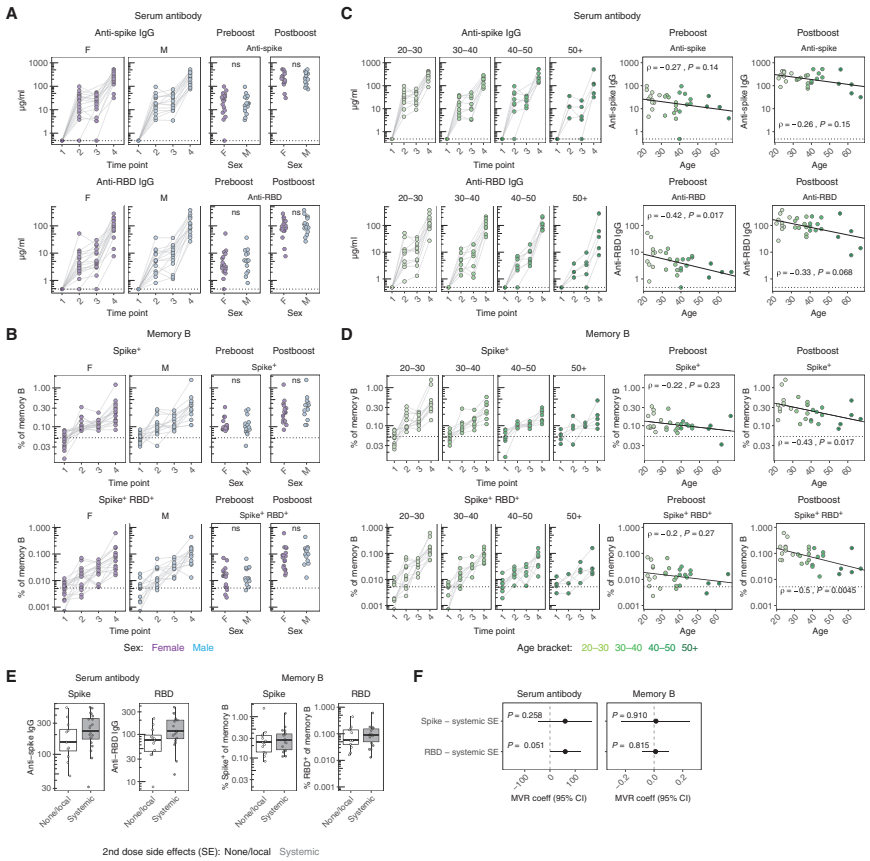

Fig. 3. Association of age and side effects with B cell responses after mRNA vaccination. (**A** and **C**) Concentration of anti-spike and anti-RBD IgG antibodies over time compared with sex (A) and age (C) in SARS-CoV-2 naïve individuals. Dotted lines indicate the LOD for the assay. (**B** and **D**) Frequency of $spike^+$ and $spike^+/RBD^+$ memory B cells over time compared with sex (B) and age (D) in SARS-CoV-2 naïve individuals. Dotted lines indicate the mean frequency of cells at baseline. Preboost indicates samples collected at time point 2 (~15 days after primary vaccination). Postboost indicates samples collected at time point 4 (~7 days after secondary vaccination). Statistics for sex were calculated using Wilcoxon test. Associations with age were calculated using Spearman rank correlation and are shown with linear trend lines. (**E**) Concentration of anti-spike and anti-RBD IgG antibodies and frequency of $spike^+$ and $spike^+/RBD^+$ memory B cells at the postboost time point compared with self-reported side effects after the second dose. Reactogenicity was categorized into either no/local symptoms or systemic symptoms. (**F**) Multivariable linear regression between antibody or memory B cell responses and side effects, controlling for sex and age. Data are represented as estimated regression coefficients with a 95% CI.

(Fig. 4A). We next performed a principal components analysis (PCA) of postboost B cell responses. Antibody and memory B cell measurements had distinct contributions to the first two principal components, with total binding antibodies and neutralizing titers primarily contributing to dimension 1 (Dim1) and memory cells primarily contributing to Dim2 (Fig. 4B). On the basis of these data, we further examined the relationship between circulating antibody responses and corresponding memory B cell responses after two doses of vaccine in a bivariate analysis. Despite strong induction of both spike- and RBD-specific antibody and memory B cells in these subjects, there was no association between the levels of postboost antibodies and B cell memory (Fig. 4C), indicating that short-term serological responses and memory B cell responses may be distinct immunological features of response to mRNA vaccination. Similarly,

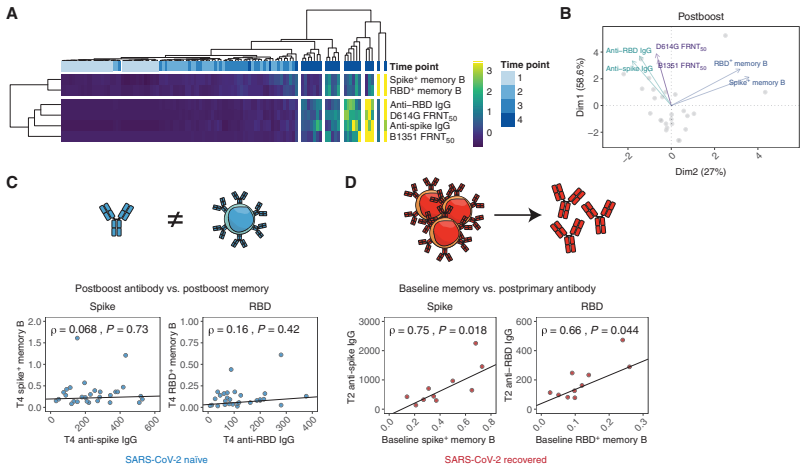

Fig. 4. Antigen-specific memory B cells were a distinct measure of vaccine efficacy and correlated to antibody recall responses. (**A**) Heatmap and hierarchical clustering of vaccine-induced antibody and memory B cell responses. (**B**) PCA and biplot of vaccine-induced antibody and memory B cell responses. (**C**) Association of postboost (time point 4) antibody levels with postboost (time point 4) antigen-specific memory B cell frequencies in SARS-CoV-2 naïve individuals. (**D**) Association of baseline (time point 1) antigen-specific memory B cell frequencies with postprimary vaccination (time point 2) antibody levels in SARS-CoV-2 recovered individuals. Illustrations in (C) and (D) represent the corresponding immune relationship. Associations between immunological parameters were calculated using Spearman rank correlation and are shown with nonparametric trend lines (Theil-Sen estimator).

prevaccine baseline antibody levels did not correlate with baseline memory B cell frequencies in SARS-CoV-2 recovered individuals (fig. S5A). We next asked which measure of humoral immunity predicted antibody recall responses after vaccination. The baseline levels of SARS-CoV-2–specific antibody correlated with the level of anti-spike but not anti-RBD antibody achieved after primary vaccine in SARS-CoV-2 recovered donors (fig. S5B). However, the baseline frequency of antigen-specific memory B cells strongly correlated with postprimary vaccination antibody levels for both spike and RBD (Fig. 4D), consistent with the notion that these prevaccination memory B cells are major contributors to the SARS-CoV-2 antibody recall response. These data highlight the importance of measuring antigen-specific memory B cells in addition to serologic antibody evaluation as an immunological correlate of vaccine-induced immunity.

Overall, we tracked antibody and antigen-specific memory B cells over time after SARS-CoV-2 mRNA vaccination and documented robust priming of antibody and memory B cell responses (Fig. 5A). Our analysis revealed key differences in vaccine-induced immune response between SARS-CoV-2 naïve and recovered subjects after the first versus second dose of vaccine. (Fig. 5B). SARS-CoV-2 naïve individuals required two doses of vaccine to achieve optimal priming of antibodies, including neutralizing antibodies to the B.1.351 strain and memory B cells. In contrast, SARS-CoV-2 recovered subjects may only require a single vaccine dose to achieve peak antibody and memory B cell responses. We also revealed age-related differences in vaccine-induction of immune responses (Fig. 5C) and highlighted the importance of memory B cells in mounting recall antibodies in SARS-CoV-2 recovered subjects (Fig. 5D).

DISCUSSION

Here, we demonstrated that mRNA vaccines to SARS-CoV-2 induced robust antibody and memory B cell responses to full-length spike and the RBD. These results are encouraging for both short- and long-term vaccine efficacy and add to our understanding of SARS-CoV-2 mRNA vaccine–induced immune responses in several ways. First, our serological data are consistent with several other recent studies (*20*, *21*, *23*, *24*, *28*, *29*), indicating robust boosting of antibody responses in SARS-CoV-2 recovered subjects after the first vaccine dose but little benefit to antibody levels after the second vaccine dose. This finding was also reflected in the observation that neutralizing titers against both D614G and the B.1.351 South African variant reached a peak after the first dose in recovered subjects. Moreover, we found a similar effect for virus-specific memory B cells, identifying a quantitative and qualitative plateau in vaccine-induced memory B cells in COVID-19 recovered subjects after the first dose of vaccine with little additional change to the memory B cell response after booster vaccination. These data suggest that only a single vaccine dose in individuals confirmed to have previously been infected with SARS-CoV-2 may be enough to induce antibody and memory B cell responses.

The data presented document key differences in immune responses associated with vaccine efficacy in SARS-CoV-2 naïve versus SARS-CoV-2 recovered individuals. However, with a study of this size designed for deep immunological analysis, it was not possible to directly address protection or true vaccine efficacy. Accordingly, larger-scale clinical studies would be necessary to fully examine the question of a one- or two-dose regimen in SARS-CoV-2 recovered

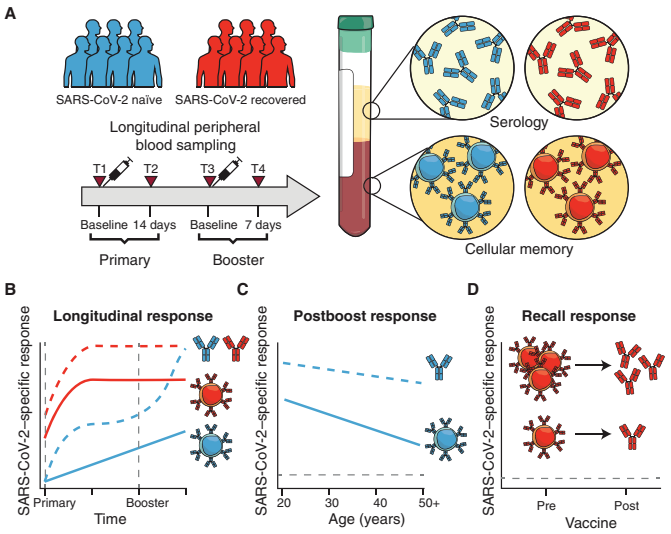

Fig. 5. Study summary and key findings. (**A**) Cohort design and objectives. Longitudinal samples were collected from SARS-CoV-2 naïve and recovered individuals and measured for both antibodies and memory B cells. (**B**) Distinct patterns of antibody and memory B cell responses to mRNA vaccination in SARS-CoV-2 naïve and recovered individuals. (**C**) Age-associated differences in antibody and memory B cell responses to mRNA vaccination. (**D**) Baseline memory B cells in SARS-CoV-2 recovered individuals contribute to recall responses after mRNA vaccination.

individuals. Our cohort also consisted of individuals who were not hospitalized during their SARS-CoV-2 infections, and it may be necessary to address this question of one versus two doses of vaccine in individuals who experienced more severe COVID-19. Moreover, there may be practical challenges to identifying SARS-CoV-2 recovered individuals based on self-reported infection or laboratory confirmed tests such as reverse transcription polymerase chain reaction (PCR) or serology. Despite these limitations, the robust boosting of both antibody and memory B cells in these subjects after one dose may have implications for vaccine distribution in settings where supply is limited.

An additional question is whether the second vaccine dose in recovered individuals has other immunological effects not reflected in overall antibody titers or memory B cell frequency and phenotype. Given the relatively short time frame of this study, future studies will be necessary to evaluate durability of immune responses in these subjects and investigate potential differences in long-term immunological memory. Our data indicate that preformed spike-binding memory B cell clones that were resampled at multiple time points did not have obvious increases in SHM, suggesting that the B cell clones boosted by mRNA vaccination in SARS-CoV-2 recovered individuals have stable SHM profiles. However, these analyses were only performed on a small number of individuals, and samples were limited to only the first few weeks after vaccination. Thus, it will be important to determine whether these clones evolve and undergo further SHM over time as occurs after natural SARS-CoV-2 infection (*9*, *30*, *31*). Even small changes in SHM may be biologically relevant, as somatically mutated clones can exhibit higher degrees of cross-protection against different mutant strains of the virus (*30*). It is also possible that other postgerminal center clones emerge later in the memory phase. Last, it is possible that booster vaccination has some beneficial effects on virus-specific T cell responses in SARS-CoV-2 recovered individuals. Given the capacity of mRNA vaccines to induce $CD4^+$ T cell responses (*32*), this topic merits further investigation.

In contrast to SARS-CoV-2 recovered subjects, SARS-CoV-2 naïve individuals demonstrated considerable benefit to antibody and memory B cell responses from the second dose of mRNA vaccine. It is possible that some of this benefit would occur over time in the absence of a second vaccine dose; however, the spike- and RBD-specific antibody levels appeared to plateau between the first and second doses of vaccine before increasing again after the second dose. In addition, only half of SARS-CoV-2 naïve individuals had neutralizing antibodies to wild-type virus, and only 2 of 25 had neutralizing antibodies to the B.1.351 variant after the first dose of vaccine, whereas nearly all subjects achieved neutralizing antibodies after dose two. Moreover, the frequency of memory B cells that were IgG^+ and the fraction that was focused on RBD both increased after the second vaccine dose, indicating an improvement in the quality of the memory B cell response. Together, these data are consistent with the need for a two-dose mRNA vaccine schedule in SARS-CoV-2 naïve individuals to achieve optimal levels of humoral immunity, including neutralizing antibodies against the B.1.351 variant.

We also observed a negative association of age with induction of B cell memory. Others have reported a negative association between age and serum antibody titers after a single mRNA vaccine dose (*28*, *29*). We found a similar trend for antibodies after two doses of mRNA vaccination, but this did not reach statistical significance for our cohort. However, the magnitude of the memory B cell response after the second dose was lower with increased age, confirming age as a key variable in mRNA vaccine–induced immunity. It remains unclear whether the age-associated effect on memory B cell induction represents a true difference in the magnitude of response or a difference in kinetics that will resolve at later time points. It is also challenging to define an exact threshold for how much immunological memory is sufficient to provide long-term protection. Although all subjects, regardless of age, had significant humoral and memory B cell responses to vaccination, these data highlight a need to further understand the age-related changes in responses to mRNA vaccination (*33*). In examining correlates of vaccine-induced immune responses, we also uncovered a trend suggesting that vaccine-induced side effects may be related to postvaccination serum antibodies, but not

memory B cells. Although more data are needed, it is possible that systemic inflammation early after vaccination could contribute to an initial induction of antibody with less of an impact on the development of memory B cells. Larger cohorts and more quantitative measures of vaccine-induced side effects may further clarify these relationships.

Last, these analyses highlight the importance of interrogating vaccine-induced memory B cell responses alongside serological analyses. Specifically, we found no relationship between postvaccination serum antibody levels and memory B cells in SARS-CoV-2 naïve subjects, indicating that antibody and memory B cell induction may be independent features of the immune response to mRNA vaccination. Previous work has found that antibodies and memory B cells correlate for some vaccines or antigens but do not correlate for many others (*34*). Current research on SARS-CoV-2 vaccines has largely focused on measuring circulating antibodies without measuring memory B cells, which are important for durability of immune memory and potential recall responses to infection or future booster. Preexisting memory B cells in SARS-CoV-2 recovered subjects correlated strongly with postvaccination antibody levels in our cohort, underscoring the immunological connection between memory B cells and antibody recall responses (*35*). Together, our findings highlight the importance of evaluating memory B cells in addition to serologies to more completely characterize humoral immunity. Although high circulating titers of neutralizing antibodies are common surrogates of protective immunity, there are many scenarios where circulating antibodies may not achieve sterilizing immunity and additional immune responses from memory cells will be necessary (*36*). For example, high-dose viral inoculums may require rapid generation of additional antibody from memory B cells. Moreover, if circulating antibodies wane over time, our data suggest that durable memory B cells are likely to provide a rapid source of protective antibody upon SARS-CoV-2 reexposure. Last, infection with variant strains that partially escape neutralization by existing circulating antibodies (*37*–*39*) might require strong memory B cell populations that can reseed germinal centers and diversify to respond to novel spike antigens (*40*).

In summary, our analysis of antibodies and cellular memory reveals distinct responses to SARS-CoV-2 mRNA vaccines based on prior history of infection. The addition of memory B cells in this analysis, both in terms of frequency and phenotype, provides complementary data that strengthen current serology-based evidence (*20*, *21*, *23*, *24*, *28*, *29*) for a single-dose vaccine schedule in COVID-19 recovered individuals. We also find associations of vaccine-induced immune responses with age and side effects, which may have relevance for future booster vaccines and public health campaigns. Thus, our study provides insight into the underlying immunobiology of mRNA vaccines in humans and may have implications for vaccination strategies in the future.

MATERIALS AND METHODS

Study design

The objective of this study was to define antigen-specific measures of humoral immunity in peripheral blood of healthy adults after SARS-CoV-2 mRNA vaccination. A secondary objective of this study was to compare antigen-specific responses with mRNA vaccination in SARS-CoV-2 naïve and recovered individuals. This study began in December 2020 and is continuing to enroll participants.

Recruitment and clinical sample collection

Forty-four individuals (33 SARS-CoV-2 naïve and 11 SARS-CoV-2 recovered) were consented and enrolled in the study with approval from the University of Pennsylvania Institutional Review Board (IRB# 844642). All participants were otherwise healthy and, based on self-reported health screening, did not report any history of chronic health conditions. Subjects were stratified on the basis of self-reported and laboratory evidence of a prior SARS-CoV-2 infection. Of the self-reported naïve subjects, one individual was found to have positive SARS-CoV-2–specific antibodies and memory B cells at baseline and was retroactively classified as SARS-CoV-2 recovered. All subjects received either Pfizer (BNT162b2) or Moderna (mRNA-1273) mRNA vaccines. Samples were collected at four time points: baseline, 2 weeks after primary immunization, day of booster immunization, and 1 week after booster immunization. Time points were chosen a priori to capture the peak antigen-specific response for primary (*41*) and secondary responses (*42*, *43*) in SARS-CoV-2 naïve individuals. Eighty to 100 ml of peripheral blood samples and clinical questionnaire data were collected at each study visit. Full cohort and demographic information is provided in fig. S1. Nonvaccinated recovered COVID-19 donors (RD) were adults with a prior positive COVID-19 PCR test by self-report who met the definition of recovery by the Centers for Disease Control (*44*).

Sample processing

Venous blood was collected into sodium heparin and EDTA tubes by standard phlebotomy. Blood tubes were centrifuged at 3000 rpm for 15 min to separate plasma. Heparin and EDTA plasma were stored at −80°C for downstream antibody analysis. Remaining whole blood was diluted 1:1 with RPMI + 1% fetal bovine serum (FBS) + 2 mM L-glutamine + 100 U of penicillin/streptomycin and layered onto SEPMATE tubes (STEMCELL Technologies) containing lymphoprep gradient (STEMCELL Technologies). SEPMATE tubes were centrifuged at 1200*g* for 10 min and the PBMC fraction was collected into new tubes. PBMCs were then washed with RPMI + 1% FBS + 2 mM L-glutamine + 100 U of penicillin/streptomycin and treated with ammonium-chloride-potassium (ACK) lysis buffer (Thermo Fisher Scientific) for 5 min. Samples were washed again with RPMI + 1% FBS + 2 mM L-glutamine + 100 U of penicillin/streptomycin, filtered with a 70-μm filter, and counted using a Countess automated cell counter (Thermo Fisher Scientific). Aliquots containing 5×10^6 PBMCs were cryopreserved in fresh 90% FBS and 10% dimethyl sulfoxide.

Detection of SARS-CoV-2–specific antibodies

Plasma samples were tested for SARS-CoV-2–specific antibody by ELISA as previously described (*45*). The estimated sensitivity of the test is 100% [95% confidence interval (CI), 89.1 to 100.0%], and the specificity is 98.9% (95% CI, 98.0 to 99.5%) (*45*). Plasmids encoding the recombinant full-length spike protein and the RBD were provided by F. Krammer (Mt. Sinai) and purified by nickel–nitrilotriacetic acid resin (Qiagen). ELISA plates (Immulon 4 HBX, Thermo Fisher Scientific) were coated with phosphate-buffered saline (PBS) or recombinant protein (2 μg/ml) and stored overnight at 4°C. The next day, plates were washed with PBS containing 0.1% Tween 20 (PBS-T) and blocked for 1 hour with PBS-T supplemented with 3% nonfat milk powder. Samples were heat inactivated for 1 hour at 56°C and diluted in PBS-T supplemented with 1% nonfat milk powder.

After washing the plates with PBS-T, 50 μl of diluted sample was added to each well. Plates were incubated for 2 hours and washed with PBS-T. Next, 50 μl of diluted goat anti-human IgG–horseradish peroxidase (HRP) (1:5000; Jackson ImmunoResearch Laboratories) or diluted goat anti-human IgM-HRP (1:1000; SouthernBiotech) was added to each well, and plates were incubated for 1 hour. Plates were washed with PBS-T before 50 μl of SureBlue 3,3′,5,5′-tetramethylbenzidine substrate (KPL) was added to each well. After 5-min incubation, 25 μl of 250 mM hydrochloric acid was added to each well to stop the reaction. Plates were read with the SpectraMax 190 microplate reader (Molecular Devices) at an optical density (OD) of 450 nm. Monoclonal antibody CR3022 was included on each plate to convert OD values into relative antibody concentrations. Plasmids to express CR3022 were provided by I. Wilson (Scripps).

SARS-CoV-2 neutralization assay

Production of vesicular stomatitis virus (VSV) pseudotypes with SARS-CoV-2 spike

293T cells plated 24 hours previously at 5×10^6 cells per 10-cm dish were transfected using calcium phosphate with 35 μg of pCG1 SARS-CoV-2 S D614G delta 18 or pCG1 SARS-CoV-2 S B.1.351 delta 18 expression plasmid encoding a codon-optimized SARS-CoV-2 S gene with an 18-residue truncation in the cytoplasmic tail (provided by S. Pohlmann). Twelve hours after transfection, the cells were fed with fresh media containing 1 mM sodium butyrate to increase expression of the transfected DNA. Twenty-four hours after transfection, the SARS-CoV-2 spike–expressing cells were infected for 2 hours with VSV-G pseudotyped VSVΔG-RFP (red fluorescent protein) at a multiplicity of infection of ~1. Virus-containing media was removed and the cells were refed with media without serum. Media containing the VSVΔG-RFP SARS-CoV-2 pseudotypes were harvested 28 to 30 hours after infection and clarified by centrifugation twice at 6000*g* then aliquoted and stored at −80°C until being used for antibody neutralization analysis.

Antibody neutralization assay using VSVΔG-RFP SARS-CoV-2

All sera were heat inactivated for 30 min at 55°C before use in neutralization assay. Vero E6 cells stably expressing TMPRSS2 were seeded in 100 μl at 2.5×10^4 cells per well in a 96-well collagen-coated plate. The next day, twofold serially diluted serum samples were mixed with VSVΔG-RFP SARS-CoV-2 pseudotype virus (100 to 300 focus-forming units per well) and incubated for 1 hour at 37°C. Also included in this mixture to neutralize any potential VSV-G carryover virus was 1E9F9, a mouse anti-VSV Indiana G, at a concentration of 600 ng/ml (Absolute Antibody, Ab01402-2.0). The serum-virus mixture was then used to replace the media on VeroE6 TMPRSS2 cells. Twenty-two hours after infection, the cells were washed and fixed with 4% paraformaldehyde before visualization on an S6 FluoroSpot Analyzer (CTL, Shaker Heights OH). Individual infected foci were enumerated, and the values compared with control wells without antibody. The focus reduction neutralization titer 50% ($FRNT_{50}$) was measured as the greatest serum dilution at which focus count was reduced by at least 50% relative to control cells that were infected with pseudotype virus in the absence of human serum. $FRNT_{50}$ titers for each sample were measured in at least two technical replicates and were reported for each sample as the geometric mean of the technical replicates.

Detection of SARS-CoV-2–specific memory B cells

Antigen-specific B cells were detected using biotinylated proteins in combination with different streptavidin (SA)–fluorophore conjugates. Biotinylated proteins were multimerized with fluorescently labeled SA for 1 hour at 4°C. Full-length spike protein (R&D Systems) was mixed with SA-BV421 (BioLegend) at a 10:1 mass ratio (e.g., 200 ng of spike with 20 ng of SA; ~4:1 molar ratio). Spike RBD (R&D Systems) was mixed with SA-APC (BioLegend) at a 2:1 mass ratio (e.g., 25 ng of RBD with 12.5 ng of SA; ~4:1 molar ratio). Biotinylated influenza HA pools were mixed with SA-PE (BioLegend) at a 6.25:1 mass ratio (e.g., 100 ng of HA pool with 16 ng of SA; ~6:1 molar ratio). Individual influenza HA antigens corresponding with the 2019 trivalent vaccine (A/Brisbane/02/2018/H1N1 and B/Colorado/06/2017; Immune Technology) were biotinylated using the EZ-Link Micro NHS-PEG4 Biotinylation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Excess biotin was subsequently removed using Zebra Spin Desalting Columns 7K MWCO (Thermo Fisher Scientific), and protein was quantified with a Pierce BCA Assay (Thermo Fisher Scientific). SA-BV711 (BD Bioscience) was used as a decoy probe without biotinylated protein to gate out cells that nonspecifically bind SA. All experimental steps were performed in a 50/50 mixture of PBS + 2% FBS and Brilliant Buffer (BD Bioscience). Antigen probes for spike, RBD, and HA were prepared individually and mixed together after multimerization with 5 μM free D-biotin (Avidity LLC) to minimize potential cross-reactivity between probes. For staining, 5×10^6 cryopreserved PBMC samples were prepared in a 96-well U-bottom plate. Cells were first stained with Fc block (BioLegend, 1:200) and Ghost 510 Viability Dye (Tonbo Biosciences, 1:600) for 15 min at 4°C. Cells were then washed and stained with 50 μl of antigen probe master mix containing 200 ng of spike-BV421, 25 ng of RBD-APC, 100 ng of HA-PE, and 20 ng of SA-BV711 decoy for 1 hour at 4°C. After incubation with antigen probe, cells were washed again and stained with anti-CD3 (BD Bioscience, 1:200), anti-CD19 (BioLegend, 1:100), anti-CD20 (BD Bioscience, 1:500), anti-CD27 (BD Bioscience, 1:200), anti-CD38 (BD Bioscience, 1:200), anti-CD71 (BD Bioscience, 1:50), anti-IgD (BD Bioscience, 1:50), anti-IgM (BioLegend, 1:200), and anti-IgG (BioLegend, 1:400). After surface stain, cells were washed and fixed in 1% paraformaldehyde overnight at 4°C. For sorting, cells were stained with spike and HA probes followed by Fc block and Ghost 510 Viability Dye as described above. Cells were then stained for surface markers with anti-CD4 (Invitrogen, 1:333.3), anti-CD8 (BioLegend, 1:66.7), anti-CD14 (BioLegend, 1:200), anti-CD19 (BD Bioscience, 1:100), anti-CD27 (BioLegend, 1:66.7), and anti-CD38 (1:200). After surface stain, cells were washed and resuspended in PBS + 2% FBS for acquisition. All antibodies and recombinant proteins are listed in tables S1 and S2.

Flow cytometry and cell sorting

Samples were acquired on a BD Symphony A5 instrument. Standardized SPHERO rainbow beads (Spherotech) were used to track and adjust photomultiplier tubes over time. UltraComp eBeads (Thermo Fisher Scientific) were used for compensation. Up to 5×10^6 cells were acquired per sample. Data were analyzed using FlowJo v10 (BD Bioscience). Antigen-specific gates were set on the basis of healthy donors stained without antigen probes [similar to an fluorescence minus one (FMO) control] and were kept the same for all experimental runs. All time points for individual subjects were run in the same experiment to minimize batch effects. The full gating strategy is shown in fig. S2. Cell sorting was performed on a BD FACSAria II instrument in low-pressure mode, using a 70-μm nozzle. SARS-CoV-2–specific memory B cells were similarly identified

as live, CD14$^-$, CD19$^+$, CD27$^+$ CD38$^{lo/int}$, and HA$^-$ spike$^+$. Cells were sorted into 1.5 DNA LoBind Eppendorf tubes containing 300 µl of cell lysis buffer (Qiagen) and stored at room temperature until nucleic acid extraction.

BCR sequencing

DNA was extracted from sorted cells using a Gentra Puregene Cell kit (Qiagen, catalog no. 158767). Immunoglobulin heavy-chain family–specific PCRs were performed on genomic DNA samples using primers in FR1 and JH as described previously (*46*, *47*). Two biological replicates were run on all samples. Sequencing was performed in the Human Immunology Core Facility at the University of Pennsylvania using an Illumina 2× 300-bp paired-end kit (Illumina MiSeq Reagent Kit v3, 600-cycle, Illumina MS-102-3003).

BCR sequence analysis

Raw reads from the Illumina MiSeq were quality controlled with pRESTO v0.6.0 (*48*) as described in (*49*). Sequences passing the quality control procedure were imported into IgBLAST v1.17.0 (*50*) for gene identification and alignment. The primer binding region (IMGT nucleotide positions 1 to 80) was replaced with *N*s and sequences beginning after IMGT position 90 were removed to avoid incorrect V gene calls and skewed SHM analysis. The remaining sequences were imported into ImmuneDB v0.29.10 (*51*) for clonal inference, lineage construction, and downstream analyses. Sequences sharing the same VH gene, JH gene, CDR3 length, and 85% amino acid homology in the CDR3 were aggregated into clones. After sequences were collapsed into clones, nonproductive sequences and clones with one copy number sequences were excluded from all downstream analysis.

Lineages were constructed within ImmuneDB as described in (*51*). Within each lineage, sequences with fewer than 10 copies across all samples in a donor were excluded to reduce the effect of sequencing error and improve fidelity. The resulting lineage structures were visualized with ete3 (*52*). Each node represents a unique sequence and the size of each node is proportional to the total copy number of the sequence. Nodes are depicted as pie charts where each wedge indicates the proportion of copies at each time point and inferred nodes are shown in black. The number next to each node represents the number of nucleotide mutations as compared with the preceding vertical node.

Data visualization and statistics

All antibody and memory B cell data were analyzed using custom scripts in R Studio. BCR sequencing data were analyzed as discussed above. Data were visualized using ggplot2 in R Studio. Boxplots represent median with interquartile range. Line plots represent means with a 95% CI. For heatmaps, data were scaled by variable (z score normalization) and cells with $z > 3.5$ were assigned a maximum value of 3.5. For PCA, data were also scaled by variable (z score normalization). Statistical tests are indicated in the corresponding figure legends. All tests were performed two sided with a nominal significance threshold of $P < 0.05$. In all cases of multiple comparisons, adjustment was performed using Holm correction. For comparisons between time points, unpaired tests were used due to missing data/samples for some participants. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. ns indicates no significant difference. Blue and red values indicate statistical comparisons within naïve or recovered groups. Black values indicate statistical comparisons between naïve or recovered groups. Source code is available upon request from the authors. All raw data are provided in table S4.

SUPPLEMENTARY MATERIALS

immunology.sciencemag.org/cgi/content/full/6/58/eabi6950/DC1
Figs. S1 to S5
Tables S1 to S4

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

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and preventing government funding of mandates. Enforcement tools included facilitating citizen lawsuits against municipal governments that introduce mandates and firing, fining, or imprisoning violators. Some states proactively created exemptions or rights of refusal for any future mandates or established protections against discrimination based on vaccination status. Legal interventions in 13 states cited lack of US Food and Drug Administration approval as a reason to impede vaccine mandates.

Discussion | Recent US state-level legal interventions to facilitate or impede vaccine mandates have had moderate success. While most interventions aimed to impede mandates, the majority were not enacted. In contrast, most of the interventions proposed to facilitate mandates were enacted. However, given the higher number of interventions aimed at impeding mandates, more were ultimately enacted than interventions to facilitate mandates. Study limitations include that the frequency of interventions that addressed mandates in multiple domains (eg, employment and education) was not analyzed, nor was the consistency of individual states' approaches to mandates. Future work should address these issues and trace the processes by which state-level legal interventions are formulated.

Breanna Fernandes
Mark Christopher Navin, PhD
Dorit Rubinstein Reiss, PhD
Saad B. Omer, MBBS, MPH, PhD
Katie Attwell, PhD

Author Affiliations: University of Western Australia, Perth, Australia (Fernandes, Attwell); Oakland University, Rochester, Michigan (Navin); Hastings College of the Law, University of California, San Francisco (Reiss); Yale Institute for Global Health, New Haven, Connecticut (Omer).

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Corresponding Author: Breanna Fernandes, University of Western Australia, Perth, 35 Stirling Hwy, Crawley, WA 6009, Australia (22981317@student.uwa.edu.au).

Author Contributions: Ms Fernandes had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Fernandes, Navin, Omer, Attwell.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Fernandes, Navin, Attwell.
Critical revision of the manuscript for important intellectual content: Fernandes, Navin, Reiss, Omer.
Statistical analysis: Fernandes.
Administrative, technical, or material support: Fernandes, Navin, Attwell.
Supervision: Attwell.

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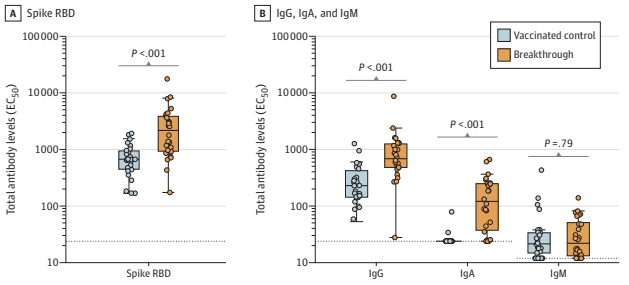
Antibody Response and Variant Cross-Neutralization After SARS-CoV-2 Breakthrough Infection

Breakthrough infections after vaccination against SARS-CoV-2 are increasingly reported, possibly due to waning of vaccine-induced antibody levels.[1] Moreover, emerging variants of concern with diminished susceptibility to vaccine-induced antibodies are responsible for most new cases.[2,3] Studies have focused on determining the rate of vaccine breakthrough based on antibody levels after standard vaccination practices.[4,5] We assessed antibody levels and variant cross-neutralization after breakthrough infection.

Supplemental content

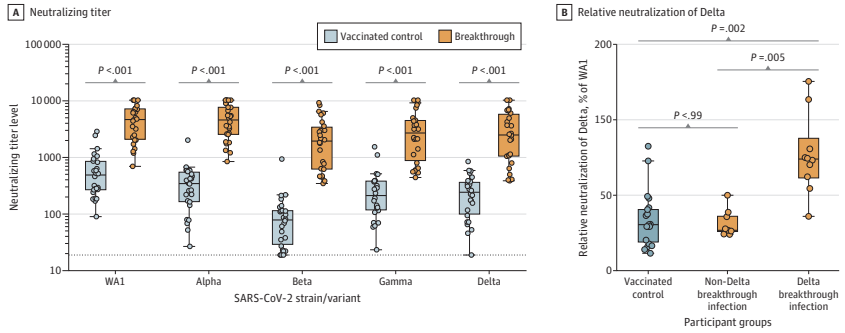
Methods | Fully vaccinated health care workers subsequently diagnosed with SARS-CoV-2 breakthrough infection based on a positive polymerase chain reaction (PCR) test result were sequentially recruited at the Oregon Health & Science University between January 31, 2021, and August 18, 2021. Only those with no history of previous infection whose test results were negative for nucleocapsid antibodies were included. Controls were fully vaccinated individuals without a breakthrough infection matched on sex, age, time between vaccine doses, and time between sample collection and most recent antigen exposure (PCR confirmation for those with breakthrough infection and final vaccine dose for controls). Full-length viral genomic sequencing was used to determine SARS-CoV-2 variant identity. Enzyme-linked immunosorbent assays were used to determine serum dilution titers with a 50% effective concentration (EC_{50}) of IgG, IgA, and IgM antibodies specific to the SARS-CoV-2 spike receptor–binding domain. Live SARS-CoV-2 neutralizing serum dilution titers were determined by 50% focus reduction neutralization tests ($FRNT_{50}$) against isolates of the original SARS-CoV-2 strain (WA1) and variants of concern (Alpha, Beta, Gamma, and Delta). Median breakthrough and control serum values were calculated in GraphPad Prism and compared with the Wilcoxon matched-pairs signed rank test with the Holm-Šídák correction. Delta-neutralizing potency was determined by comparing Delta- and WA1-neutralizing titers for sequence-confirmed Delta variant breakthrough cases, non-Delta breakthrough cases, and controls using the Kruskal-Wallis test with Dunn correction. Statistical significance was defined as a 2-tailed $P < .05$. Additional laboratory methods are provided in the Supplement. The Oregon Health & Science University institutional review board approved this study. Written informed consent was obtained.

Figure 1. SARS-CoV-2 Spike Receptor-Binding Domain (RBD)–Specific Antibody Levels After Vaccination and Breakthrough Infection



Enzyme-linked immunosorbent assay measurement of serum dilution titers with a 50% effective concentration (EC_{50}) of SARS-CoV-2 spike RBD-binding antibodies. The dotted lines indicate the assay limits of detection. Two-tailed *P* values were determined using the Wilcoxon matched-pairs signed rank test with the Holm-Šídák multiple comparison correction. Box plots were generated using the Tukey method. The large box displays the median and IQR. The error bars indicate 1.5 times the IQR or the furthest outlier, whichever is closer to the median. All individual data points are displayed as filled circles.

Figure 2. Live SARS-CoV-2 Variants Neutralization After Vaccination and Breakthrough Infection



Live SARS-CoV-2 neutralization by focus-forming assay. A, The dotted line indicates the assay limit of detection. Two-tailed *P* values were determined using the Wilcoxon matched-pairs signed rank test with the Holm-Šídák multiple comparison correction. B, Participants with inconclusive sequencing information were excluded from this analysis. Two-tailed *P* values were determined using the Kruskal-Wallis test with the Dunn multiple comparison correction. Box plots were generated using the Tukey method. The large box displays the median and IQR. The error bars indicate 1.5 times the IQR or the furthest outlier, whichever is closer to the median. All individual data points are displayed as filled circles.

Results | Twenty-six participants with breakthrough infections (mean age, 38 years; 20 [77%] women; 24 [92%] were vaccinated with BNT162b2, sampled a median 28 days after PCR date and 213.5 days after final vaccination; 21 [81%] with mild symptoms) were matched to 26 controls (mean age, 39 years; 21 [81%] women; 26 [100%] were vaccinated with BNT162b2, sampled a median 28 days after final vaccination). Total receptor-binding domain–specific immunoglobulin increased in participants with breakthrough infection with a median EC_{50} of 2152 (95% CI, 961-3596) compared with 668 (95% CI, 473-892) in controls (322% increase; $P < .001$) (**Figure 1**A). Median serum dilutions increased for both IgG and IgA. For example, the median IgA EC_{50} after breakthrough infection was 120 (95% CI, 44-246), compared with 24 (95% CI, 24-24) for controls (502% increase; $P < .001$). IgM levels were not significantly different between groups (Figure 1B).

Among sequence-confirmed breakthrough cases, 10 were Delta and 9 were non-Delta infections. Among breakthrough cases, the median $FRNT_{50}$ against WA1 was 4646 (95% CI, 2283-7053) vs 489 (95% CI, 272-822) for controls (950% increase; $P < .001$). $FRNT_{50}$ results for Alpha, Beta, and Gamma variants are shown in **Figure 2**A. In breakthrough cases, median $FRNT_{50}$ against the Delta variant was 2482 (95% CI, 1072-4923), compared with 243 (95% CI, 118-336) for controls (1021% increase; $P < .001$) (Figure 2A). Sera from Delta breakthrough cases showed improved potency against the Delta variant at 99% (95% CI, 73-151) of WA1 neutralization for each participant, compared with 36% (95% CI, 33-52) for non-Delta cases and 41% (95% CI, 24-56) for controls (Figure 2B).

Discussion | Results of this study showed substantial boosting of humoral immunity after breakthrough infection, despite predominantly mild disease. Boosting was most notable for IgA, possibly due to the differences in route of exposure between vaccination and natural infection. In addition, breakthrough sera demonstrated improved variant cross-neutralization, and Delta breakthrough infections in particular exhibited improved potency against Delta vs WA1, suggesting that the protective immune response may be broadened through development of variant boosters with antigenic inserts matching the emerging SARS-CoV-2 variants. Limitations of this study include the small number of samples and the difference in time from initial vaccination to serum collection between the breakthrough and control groups, which emerging evidence suggests may contribute to the development of variant cross-neutralizing antibody responses.[6]

Timothy A. Bates, BSc
Savannah K. McBride, BSc
Bradie Winders, CNA
Devin Schoen, BSc
Lydie Trautmann, EngD, PhD
Marcel E. Curlin, MD
Fikadu G. Tafesse, PhD

Author Affiliations: Department of Molecular Microbiology & Immunology, Oregon Health & Science University, Portland (Bates, McBride, Tafesse); Division of Infectious Diseases, Oregon Health & Science University, Portland (Winders, Schoen, Curlin); Vaccine and Gene Therapy Institute, Oregon Health & Science University, Portland (Trautmann).

Corresponding Author: Fikadu G. Tafesse, PhD, Department of Molecular Microbiology & Immunology, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239 (tafesse@ohsu.edu).

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Concept and design: Bates, McBride, Winders, Curlin, Tafesse.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Bates.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Bates, McBride, Schoen.
Obtained funding: Curlin, Tafesse.
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Supervision: Curlin, Tafesse.

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COMMENT & RESPONSE

Effect of *Lactobacillus rhamnosus* GG on Incident Pneumonia in Critically Ill Patients

To the Editor The recent trial[1] investigating the effect of probiotics on the risk of ventilator-associated pneumonia (VAP) in critically ill patients found no difference in the primary outcome of incidental VAP and 20 other prespecified secondary outcomes between patients receiving probiotics and those receiving placebo. Based on these findings, the authors recommended against the use of *Lactobacillus rhamnosus* GG for critically ill patients. However, we have several concerns.

First, although the baseline characteristics between the probiotic and placebo groups were similar, use of proton pump inhibitors, histamine 2 receptor antagonists, immunosuppressants, corticosteroids, and paralytic agents was not reported. All of these medications may increase the risk of VAP among critically ill patients.[2-4] Proton pump inhibitors and histamine 2 receptor antagonists both may cause an imbalance in the digestive tract, which can lead to microbiome dysbiosis. Therefore, we believe this study[1] should have adjusted for these commonly used medications in critically ill patients.

Second, we agree with the study's Limitations section about the definition of VAP and appreciate the inclusion of several definitions of VAP. However, more than one-third of patients in this study were admitted with respiratory diagnosis and approximately 60% had pneumonia on intensive care unit (ICU) admission. Therefore, it is difficult to differentiate incident VAP from prevalent pneumonia. We believe that use of ventilator-associated events may be a better diagnostic strategy to overcome some of the limitations of conventional VAP definitions, which include their complexity and subjectivity and the incomplete capture of mechanical ventilation–associated respiratory conditions.

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CORRESPONDENCE

Plasma Neutralization of the SARS-CoV-2 Omicron Variant

TO THE EDITOR: The newly emerged B.1.1.159 (omicron) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1] has a large number of changes — 32 — in its spike protein relative to that of the original virus (Wuhan-hu-1), particularly in the receptor-binding domain and the N-terminal domain, the primary targets of neutralizing antibodies. Previously, we showed that approximately 20 changes introduced into a synthetic polymutant spike protein (PMS20) are sufficient for substantial evasion of the polyclonal neutralizing antibodies elicited in the majority of persons who have recovered from coronavirus disease 2019 (Covid-19) or have received two doses of an mRNA vaccine.[2] Of note, several changes in the PMS20 spike protein are the same as or similar to changes in the omicron variant (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

We measured neutralizing antibody titers against Wuhan-hu-1, PMS20, and omicron spike pseudotypes in 169 plasma specimens from 47 persons with diverse exposures to SARS-CoV-2 antigens through infection, vaccination, or both (see Supplementary Methods and Tables S1, S2, and S3).[3-5] In plasma specimens obtained at approximately 1 month and 6 months after infection from persons who had recovered from Covid-19, the 50% neutralization titer (NT_{50}) values were a mean (±SD) of 60±47 and 37±27 times lower for PMS20 than for Wuhan-hu-1, respectively, and 58±51 and 32±23 times lower for omicron than for Wuhan-hu-1 (Fig. S2A and S2B). Similarly, plasma specimens obtained from different persons in the same cohort 1 year after infection had NT_{50} values that were 34±24 times lower for PMS20 and 43±23 times lower for omicron than for Wuhan-hu-1 (Fig. S2C).

In plasma specimens from persons who had received two doses of an mRNA vaccine (BNT162b2 [Pfizer–BioNTech] or mRNA-1273 [Moderna]) 1.3 months before sampling, the NT_{50} values were 187±24 times lower for PMS20 and 127±66 times lower for omicron than for Wuhan-hu-1 (Fig. S3A). At 5 months after vaccination, the neutralization potency was 58±23 times lower for PMS20 and 27±17 times lower for omicron (Fig. S3B). Many plasma specimens from recipients of the single-dose Ad26.COV2.S vaccine (Johnson & Johnson–Janssen), obtained 1 or 5 months after vaccination, lacked detectable neutralizing activity against PMS20 or omicron (Fig. S3C and S3D), which precluded a meaningful quantitative assessment of variant-specific differences.

Of note, however, vaccination of persons who had recovered from Covid-19 or administration of a third dose of an mRNA vaccine to vaccinated persons at least 6 months after the second dose of an mRNA vaccine led to a substantial gain in neutralizing activity against PMS20 and omicron (Fig. S4). Specifically, after vaccination in persons who had previously been infected with SARS-CoV-2, the NT_{50} values were 238 times, 214 times, and 154 times greater for Wuhan-hu-1, PMS20, and omicron pseudotypes, respectively, than the prevaccination convalescent-phase titers in the same persons (Fig. 1A). For those who had received two doses of an mRNA vaccine approximately 6 months earlier and then received a third dose of an mRNA vaccine approximately 1 month before sampling, the NT_{50} values after the booster dose were 26 times greater for Wuhan-hu-1, 35 times greater for PMS20, and 38 times greater for omicron (Fig. 1B). Neutralizing titers against omicron were substantial (ranging from 1411 to 56,537) in all persons who had had Covid-19 and were then vaccinated and in those who had received three doses of an mRNA vaccine, but titers were low or undetectable in many unvaccinated persons who had had Covid-19 and in recipients of only two doses of an mRNA vaccine (Fig. 1).

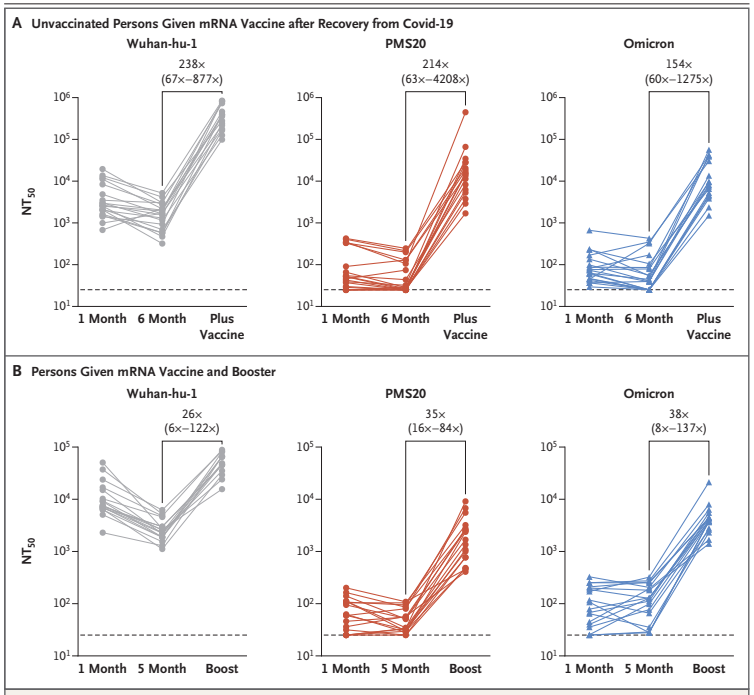

Figure 1. Wuhan-hu-1, PMS20, and Omicron Plasma Neutralizing Titers.

Panel A shows the trajectories of NT_{50} values against Wuhan-hu-1, polymutant spike protein (PMS20), and omicron pseudotypes in previously unvaccinated persons who had recovered from Covid-19, measured approximately 1 month (mean ±SD, 41±12 days) and 6 months (194±12 days) after infection and then at approximately 1 year (360±15 days) after infection, which corresponded to 41±21 days after vaccination ("plus vaccine") (see Table S2). Panel B shows the trajectories of NT_{50} values against Wuhan-hu-1, PMS20, and omicron pseudotypes in persons who had received an mRNA vaccine, measured 1 month (42±19 days) and 5 months (165±33 days) after the second dose of an mRNA vaccine and at 30±18 days after the third dose ("boost") that was administered at least 6 months after the second dose.

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Although these findings indicate that the omicron variant shows an unprecedented degree of neutralizing antibody escape, they also suggest that boosting and promoting affinity maturation of antibodies in persons who have previously been infected or vaccinated,[4,5] with the use of existing Wuhan-hu-1–based vaccine immunogens, will provide additional protection against infection with the omicron variant and subsequent disease.

Fabian Schmidt, Ph.D.
Frauke Muecksch, Ph.D.
Yiska Weisblum, Ph.D.
Justin Da Silva, M.Sc.
Eva Bednarski, B.Sc.
Alice Cho, Ph.D.
Zijun Wang, M.D., Ph.D.
Christian Gaebler, M.D.
Marina Caskey, M.D.
Michel C. Nussenzweig, M.D., Ph.D.
Theodora Hatziioannou, Ph.D.
Paul D. Bieniasz, Ph.D.
Rockefeller University
New York, NY
pbieniasz@rockefeller.edu

Drs. Schmidt and Muecksch contributed equally to this letter.

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Five-Year Outcomes of the Partial Oral Treatment of Endocarditis (POET) Trial

TO THE EDITOR: Step-down therapy with oral antibiotics has shown efficacy in some complex infectious diseases, including bone and joint infections[1] and endocarditis,[2] but data on longer-term outcomes are needed.[3] In the Partial Oral Treatment of Endocarditis (POET) trial,[2] step-down therapy with oral antibiotics after clinical stabilization of patients with endocarditis on the left side of the heart was shown to be noninferior to continued intravenous antibiotic therapy after 6 months (primary trial outcome),[2] and no indications of treatment failure after 3 years were observed.[4] Here we report the outcome of the POET trial more than 5 years after randomization (details of the trial design are provided in the protocol, available with the full text of this letter at NEJM.org).

Patients in stable condition who had endocarditis on the left side of the heart caused by streptococci, *Enterococcus faecalis*, *Staphylococcus aureus*, or coagulase-negative staphylococci were randomly assigned to continue treatment with intravenous antibiotics (199 patients) or to shift to step-down treatment with oral antibiotics (201 patients) after at least 10 days of initial treatment with intravenous antibiotics. After undergoing randomization, patients in the group that received intravenous treatment remained hospitalized until the antibiotic treatment was completed (i.e., a median of 19 days [interquartile range, 14 to 25]). Patients who received step-down treatment with oral antibiotics were discharged after a median of 3 days (interquartile range, 1 to 10) after completion of the initial treatment with intravenous antibiotics. The primary outcome was a composite of death from any cause, unplanned cardiac surgery, embolic events, and relapse of positive blood cultures after 6 months.

In this post hoc analysis, patients were followed from randomization until July 10, 2020, or until death. Longer-term follow-up was performed

CORRESPONDENCE

9

SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons

TO THE EDITOR: During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, several new viral variants have emerged, leading to the virus becoming more contagious. However, efficient immune escape has not been observed, and vaccines have remained effective. Most recently, the B.1.1.529 (omicron) variant has been described, which the World Health Organization classified as a variant of concern on November 26, 2021.[1]

The omicron variant is characterized by a large number of mutations, with 26 to 32 changes in the spike (S) protein.[2] Given that many of these mutations are in regions that are known to be involved in immune escape, we studied whether serum samples obtained from persons who had been vaccinated against SARS-CoV-2 or who had recovered from SARS-CoV-2 infection (i.e., convalescent) would be able to neutralize the omicron variant. The observation that the omicron variant is more likely than previous variants to cause reinfection suggests some level of immune escape.[3]

We obtained serum samples from persons who had been infected with the B.1.1.7 (alpha), B.1.351 (beta), or B.1.617.2 (delta) variant of SARS-CoV-2 and from persons who had received two doses of the mRNA-1273 vaccine (Spikevax, Moderna), the ChAdOx1-S vaccine (also known as ChAdOx1 nCoV-19; Vaxzevria, AstraZeneca), or the BNT162b2 vaccine (Comirnaty, Pfizer–BioNTech) or had received heterologous vaccination (i.e., one dose each) with the ChAdOx1-S and BNT162b2 vaccines. For all serum samples, we determined titers of neutralizing antibodies against the alpha, beta, delta, and omicron variants using a focus-forming assay with replication-competent SARS-CoV-2 viruses, as described previously.[4] We also obtained serum samples from persons who had been infected and were subsequently vaccinated (convalescent–vaccinated) or had been vaccinated and had subsequent breakthrough infection (vaccinated–convalescent). We analyzed neutralizing antibody titers against the delta and omicron variants in these samples.

A total of 10 participants had been infected with the alpha variant, 8 with the beta variant, and 7 with the delta variant. Ten participants had received two doses of the mRNA-1273 vaccine, 10 the ChAdOx1-S vaccine, and 20 the BNT162b2 vaccine; 20 participants had received heterologous vaccination with the ChAdOx1-S and BNT162b2 vaccines. In addition, 5 participants had been infected and subsequently received one or two doses of the BNT162b2 vaccine, and 5 had been vaccinated with two doses of the mRNA-1273, ChAdOx1-S, or BNT162b2 vaccine and subsequently had breakthrough infection. The characteristics of the participants are shown in Tables S1 through S3 in the Supplementary Appendix, available with the full text of this letter at NEJM.org.

Serum samples from vaccinated persons neutralized the omicron variant to a much lesser extent than any other variant analyzed (alpha, beta, or delta) (Fig. 1 and Table S4). We found some cross-neutralization of the omicron variant in samples obtained from persons who had received either homologous BNT162b2 vaccination or heterologous ChAdOx1-S–BNT162b2 vaccination but not in samples from persons who had received homologous ChAdOx1-S vaccination. We did not find neutralizing antibodies against the omicron variant in serum samples obtained 4 to 6 months after receipt of the second dose of the mRNA-1273 vaccine. However, in this group, the interval between receipt of the second dose and sampling was longer than for the other vaccination-regimen groups, for which serum samples were obtained only 1 month after receipt of the second dose. We did not analyze serum samples from persons who had received a third dose of vaccine. Serum samples that were obtained from convalescent participants largely did not neutralize the omicron variant, although cross-neutralization was

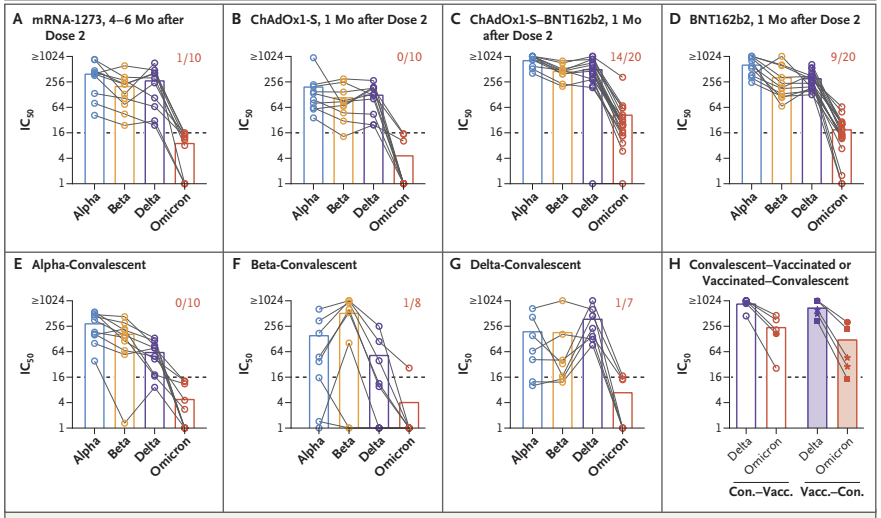

Figure 1. Neutralization of the B.1.1.529 (Omicron) Variant as Compared with Other Variants of Concern.

Serum samples were obtained from participants who had received two doses of the mRNA-1273 vaccine (Panel A), two doses of the ChAdOx1-S vaccine (Panel B), heterologous ChAdOx1-S–BNT162b2 vaccination (Panel C), or two doses of the BNT162b2 vaccine (Panel D) or who had recovered from infection (i.e., convalescent) with the B.1.1.7 (alpha) variant (Panel E), the B.1.351 (beta) variant (Panel F), or the B.1.617.2 (delta) variant (Panel G). Samples were analyzed for 50% neutralization titers (IC_{50}) against the alpha (blue), beta (orange), delta (purple), and omicron (red) variants. Bars indicate means, and symbols individual serum samples. Samples from the same participant are connected by lines. The dashed line in each panel indicates the limit of detection. The numbers in Panels A through G indicate the proportion of serum samples that were positive (>1:16) for the omicron variant. Serum samples from participants who had been infected and were subsequently vaccinated (convalescent–vaccinated; open bars) or who had been vaccinated and subsequently had breakthrough infection (vaccinated–convalescent; shaded bars) were analyzed for IC_{50} against the delta and omicron variants (Panel H). In the left part of Panel H (convalescent–vaccinated), open circles indicate participants who received a single dose of the BNT162b2 vaccine after infection, and closed circles those who received two doses of the BNT162b2 vaccine; in the right part (vaccinated–convalescent), closed circles indicate participants who had been vaccinated with two doses of the BNT162b2 vaccine before infection, stars those who had been vaccinated with two doses of the ChAdOx1-S vaccine, and squares those who had been vaccinated with two doses of the mRNA-1273 vaccine.

observed against other variants. However, 9 of the 10 serum samples that were obtained from convalescent–vaccinated or vaccinated–convalescent participants were able to neutralize the omicron variant, although to a lesser degree than the delta variant.

The omicron variant has already become the dominant variant in many countries and is causing considerable illness and death, although possibly to a somewhat lesser extent than previous variants. Although receipt of a third dose (booster) of the BNT162b2 vaccine may increase the level of cross-neutralizing antibodies to the omicron variant,[5] on the basis of the data from the present study, the rapid development of new, variant-adapted vaccines is warranted.

Annika Rössler, M.Sc.
Lydia Riepler, M.Sc.
David Bante, M.D.
Dorothee von Laer, M.D.
Janine Kimpel, Ph.D.

Medical University of Innsbruck
Innsbruck, Austria
janine.kimpel@i-med.ac.at

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Letters

RESEARCH LETTER

Immunogenicity of Extended mRNA SARS-CoV-2 Vaccine Dosing Intervals

Standard dosing intervals for BNT162b2 and mRNA-1273 SARS-CoV-2 vaccines are 21 and 28 days, respectively.[1] Data suggest improved effectiveness of ChAdOx1 adenoviral[2] and other nonreplicating vaccines[3] with increased dosing intervals, but little data exist for mRNA vaccines. This study investigated the immunogenicity of extended mRNA vaccine dosing intervals.

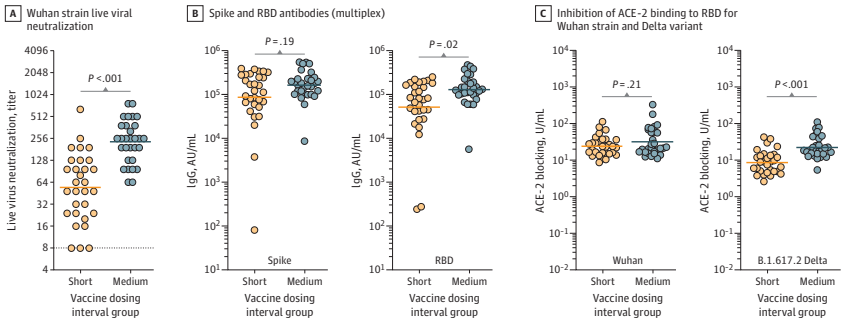
Supplemental content

Methods | The COVID-19 Occupational Risks, Seroprevalence and Immunity among Paramedics in Canada cohort study (approved by the University of British Columbia and University of Toronto research ethics boards) recruited Canadian paramedics (January 25 to July 14, 2021), with written consent. Participants who provided a blood sample at enrollment or between 170 to 190 days after the first dose and had received 2 mRNA vaccine doses were eligible for this analysis. Participants with documented COVID-19 were excluded.

We relied on observed variability of vaccine intervals and timing of enrollment relative to vaccination to select participants with different vaccine intervals and performed 2 separate investigations, with different approaches to timing of blood samples. The first investigation compared antibody levels at comparable time intervals after the second dose. For the short (≤28 days) vs medium (42-49 days) vaccine dosing interval comparison, 30 samples (collected at enrollment) from each group were selected based on similar second vaccine-to-sample-collection intervals and were matched by vaccine type, age, sex, and comorbidities (eAppendix in the Supplement). The second investigation compared antibody levels sampled at a standardized interval (170-190 days) after the first dose. For the short (≤36 days) vs long (100-120 days) comparison, 30 samples from each group were selected, matching by the same characteristics.

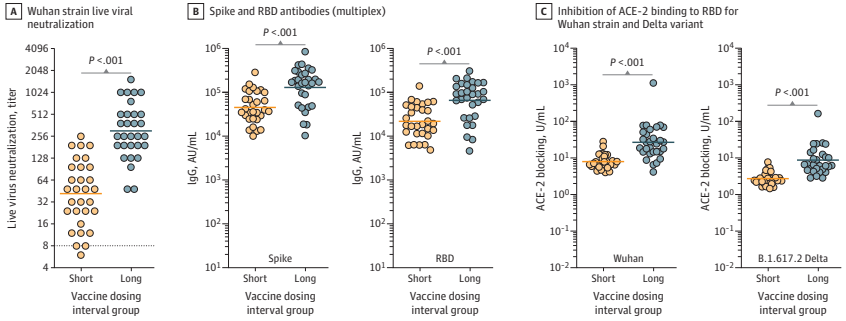
The primary outcome was the reciprocal of neutralizing antibody titers against a live Wuhan strain (eAppendix in the Supplement). Secondary outcomes included IgG antibodies to the spike protein and receptor-binding domain (RBD) using a multiplex assay (V-PLEX COVID-19 Coronavirus Panel 2 [IgG] Kit; Meso Scale Diagnostics); antibodies to spike protein using a monoplex assay (Elecsys Anti-SARS-CoV-2 S assay; Roche); and inhibition of angiotensin-converting enzyme 2 (ACE-2) binding to RBD from Wuhan, Alpha, Beta, Delta, and Gamma variants (V-PLEX COVID-19 Coronavirus Panel 11 [ACE2] Kit).

Figure 1. Comparison of Serological Outcomes in Paramedics Who Received Short (≤28 Days) vs Medium (42-49 Days) mRNA Vaccine Dosing Intervals



The solid lines indicate the geometric mean. *P* values were derived from the Wilcoxon matched-pair signed rank test. A, Reciprocal of live viral Wuhan strain neutralization titers, expressed as highest serum dilution able to block viral cytotoxicity (geometric mean, 54.6 [geometric SD {GSD}, 3.0] for the short group vs 230.8 [GSD, 2.0] for the medium group). The dashed line indicates the lower limit of detection with values below set at 1:4. B, The antibody IgG concentration for the multiplex spike was 87 292 arbitrary units (AU)/mL (SD, 5.4 AU/mL) for the short group vs 163 167 AU/mL (SD, 2.2 AU/mL) for the medium group and for the receptor-binding domain (RBD) was 51 753 AU/mL (SD, 5.3 AU/mL) for the short group vs 128 987 U/mL (SD, 2.3 U/mL) for the medium group. C, Inhibition of angiotensin-converting enzyme 2 (ACE-2) binding to the SARS-CoV-2 receptor-binding domain for the Wuhan strain was 24.0 U/mL (SD, 1.9 U/mL) for the short group vs 31.9 U/mL (SD, 2.4 U/mL) for the medium group; for the Delta variant it was 8.64 U/mL (SD, 2.1 U/mL) for the short group vs 22.3 U/mL (SD, 2.0 U/mL) for the medium group.

Figure 2. Comparison of Serological Outcomes in Paramedics Who Received Short (≤36 Days) vs Long (100-120 Days) mRNA Vaccine Dosing Intervals



The solid lines indicate the geometric mean. *P* values were derived from the Wilcoxon matched-pair signed rank test. A, Reciprocal of live viral Wuhan strain neutralization titers, expressed as highest serum dilution able to block viral cytotoxicity (geometric mean, 41.8 [geometric SD {GSD}, 2.8] for the short group vs geometric mean, 302.3 [GSD, 2.4] for the long group). The dashed line indicates the lower limit of detection with values below set at 1:4. B, The antibody IgG concentration for the multiplex spike was 45 155 arbitrary units (AU)/mL (SD, 2.3 AU/mL) for the short group vs 129 299 AU/mL (SD, 2.8 AU/mL) for the long group. The receptor-binding domain (RBD) was 22 071 AU/L (SD, 2.4 AU/L) vs 66 022 AU/L (SD, 2.9 AU/L). C, Inhibition of angiotensin-converting enzyme 2 (ACE-2) binding to SARS-CoV-2 receptor binding domain for the Wuhan strain was 7.9 U/mL (SD,1.6 U/mL) vs 26.8 U/mL (SD, 2.9 U/mL) and for the Delta variant, 2.7 U/mL (SD, 1.5 U/mL) vs 8.7 U/mL (Sd, 2.4 U/mL).

Outcomes were reported as geometric mean (geometric SD [GSD]) compared with the Wilcoxon matched-pair signed rank test using IBM SPSS. A 2-sided $P < .05$ was considered statistically significant.

Results | For the first investigation, the mean age for the short (dosing interval range, 18-28 days) group was 39 years (43% women); 70% received BNT162b2 and 30% mRNA-1273; for the medium (range, 42-49 days) group, the mean age was 41 years (47% women); 60% received BNT162b2 and 40% mRNA-1273. Comparing immunogenicity based on time after the second vaccine dose (matched at a mean of 56 days [SD, 26 days]), the viral neutralization geometric mean was 54.6 (GSD, 3.0) for the short group vs 230.8 (GSD, 2.0) for the medium group ($P < .001$). Spike and RBD IgG antibodies measured with the multiplex assay are presented in **Figure 1**. The spike antibody concentrations measured using the monoplex assay for the short group were 1697 U/mL (GSD, 1.7 U/mL) vs 2476 U/mL (GSD, 1.0 U/mL) for the medium group ($P < .001$). The ACE-2 inhibition for the Beta variant was 11.2 U/mL (GSD, 1.6 U/mL) for the short group vs 14.7 U/mL (GSD, 1.8 U/mL) for the medium group ($P = .04$). See Figure 1 for the Delta variant results. The comparisons of the Wuhan, Alpha, and Gamma variants were not statistically significant.

For the second investigation, the mean age was 41 years (60% women); 87% received BNT162b2 and 13% mRNA-1273 for both the short (range, 21-36 days) and long (range, 102-118 days) groups. Comparing immunogenicity based on time after the first vaccine dose (mean, 179 days [SD, 4.0 days] for the short group and 180 days [SD, 5.7 days] for the long group), the viral neutralization geometric mean was 41.8 (GSD, 2.8) for the short group vs 302.3 (GSD, 2.4) for the long group ($P < .001$). The multiplex IgG antibodies are presented in **Figure 2**. For the short vs long groups, the geometric mean monoplex spike antibodies were 928.4 U/mL (GSD, 2.1 U/mL) vs 1154 U/mL (GSD, 5.0 U/mL; $P = .002$). The geometric means for ACE-2 inhibition for the Alpha variant were 7.7 U/mL (GSD, 1.7 U/mL) vs 22.8 U/mL (GSD, 2.3 U/mL; $P < .001$); for the Beta variant, 5.4 U/mL (GSD, 3.1 U/mL) vs 15.5 U/mL (GSD, 2.0 U/mL; $P < .001$), and for the Gamma variant, 4.9 U/mL (GSD, 1.5 U/mL) vs 14.3 U/mL (GSD, 2.1 U/mL; $P < .001$). Results for the Wuhan and Delta variants are presented in Figure 2.

Discussion | Longer mRNA vaccine dosing intervals demonstrated improved immunogenicity, which was consistent when responses were measured based on timing of the first or second dose. These data suggest that extending dosing intervals may be particularly advantageous against the Delta variant.

A delayed second-dose strategy could yield faster partial protection to a larger proportion of the population when vaccine supplies are limited. Modeling studies have estimated overall decreased mortality with delayed second doses when accounting for partial protection provided after 1 dose, even without taking into consideration the potential benefits of delayed second doses on long-term vaccine effectiveness.[4,5] However, the trade-off of lower individual immune protection after 1 dose may be unfavorable in at-risk groups or settings where COVID-19 prevalence is high.

Limitations include lack of randomization, small sample size, and focus on middle-aged adults. Although antibody neutralization correlates with disease protection,[6] studies

should validate whether extending vaccine dosing intervals leads to more sustained vaccine protection.

Brian Grunau, MD, MHSc
David M. Goldfarb, MD
Michael Asamoah-Boaheng, MPhil
Liam Golding, BSc
Tracy L. Kirkham, PhD
Paul A. Demers, PhD
Pascal M. Lavoie, MDCM, PhD

Author Affiliations: Department of Emergency Medicine, University of British Columbia, Vancouver, Canada (Grunau); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada (Goldfarb); Faculty of Medicine, Memorial University of Newfoundland, St John's, Canada (Asamoah-Boaheng); Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, Canada (Golding); Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada (Kirkham, Demers); Department of Pediatrics, University of British Columbia, Vancouver, Canada (Lavoie).

Corresponding Author: Brian Grunau, MD, MHSc, British Columbia Resuscitation Research Collaborative, 1190 Hornby St, Fourth Floor, Vancouver, BC V6Z 2K5, Canada (brian.grunau@ubc.ca).

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Concept and design: Grunau, Goldfarb, Asamoah-Boaheng, Kirkham, Demers, Lavoie.
Acquisition, analysis, or interpretation of data: Grunau, Asamoah-Boaheng, Golding, Kirkham, Demers, Lavoie.
Drafting of the manuscript: Grunau, Asamoah-Boaheng, Lavoie.
Critical revision of the manuscript for important intellectual content: Goldfarb, Asamoah-Boaheng, Golding, Kirkham, Demers, Lavoie.
Statistical analysis: Asamoah-Boaheng, Lavoie.
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Multisystem Inflammatory Syndrome in Children by COVID-19 Vaccination Status of Adolescents in France

COVID-19 mRNA vaccine immunogenicity and effectiveness are well established in adolescents.[1] However, the effect of vaccination on multisystem inflammatory syndrome in children (MIS-C),[2] a severe complication associated with SARS-CoV-2,[3] has not yet been described. Summer 2021 in France was marked by both a fourth wave of COVID-19 cases due to the Delta variant, with a peak in August 2021, and by the recommendation of the French Public Health Agency to vaccinate children aged 12 years or older. We estimated the risk of MIS-C among adolescents by COVID-19 vaccination status during September 2021 and October 2021.

Methods | All pediatric patients diagnosed with MIS-C according to World Health Organization criteria and admitted to 1 of the 41 French pediatric intensive care units (PICUs) between September 1, 2021, and October 31, 2021, were included in this study. In addition, all patients with MIS-C who were not admitted to a PICU and mandatorily reported to the French Public Health Agency[4] during this period were included.

Data regarding age, sex, admission to a PICU, and vaccination status of patients aged 12 to 18 years (hereafter referred to as *adolescents*) were recorded.

To account for the increasing number of adolescents vaccinated over time, including during the period in which MIS-C cases were measured, hazard ratios (HRs) of unvaccinated vs vaccinated adolescents with at least 1 dose of vaccine were estimated using a Cox proportional hazards model. Given the delays between vaccine injection and immune response and between SARS-CoV-2 infection and MIS-C onset, 3 sensitivity analyses were performed in which adolescents were considered vaccinated at least 14, at least 28, and at least 42 days after the first vaccine dose. The delay of more than 42 days covers the 28 days between the first and second injection and 2 additional weeks to achieve full immunity. Data describing vaccination status per day are available from https://solidarites-sante.gouv.fr/grands-dossiers/vaccin-covid-19/article/le-tableau-de-bord-de-la-vaccination.

All statistical analyses were performed with Stata, version 16.1 (StataCorp), and a 2-sided $P < .05$ was considered statistically significant.

This study was approved as a medical registry assessment without a requirement for patient consent by the French Advisory Committee on Information Processing in Health Research.

Results | On June 15, 2021, the beginning of the adolescent COVID-19 vaccination campaign, 2.2% of the 4 989 013

Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002)

Amy Flaxman, Natalie G Marchevsky, Daniel Jenkin, Jeremy Aboagye, Parvinder K Aley, Brian Angus, Sandra Belij-Rammerstorfer, Sagida Bibi, Mustapha Bittaye, Federica Cappuccini, Paola Cicconi, Elizabeth A Clutterbuck, Sophie Davies, Wanwisa Dejnirattisai, Christina Dold, Katie J Ewer, Pedro M Folegatti, Jamie Fowler, Adrian V S Hill, Simon Kerridge, Angela M Minassian, Juthathip Mongkolsapaya, Yama F Mujadidi, Emma Plested, Maheshi N Ramasamy, Hannah Robinson, Helen Sanders, Emma Sheehan, Holly Smith, Matthew D Snape, Rinn Song, Danielle Woods, Gavin Screaton, Sarah C Gilbert*, Merryn Voysey*, Andrew J Pollard*, Teresa Lambe*, and the Oxford COVID Vaccine Trial group*

Summary

Background COVID-19 vaccine supply shortages are causing concerns about compromised immunity in some countries as the interval between the first and second dose becomes longer. Conversely, countries with no supply constraints are considering administering a third dose. We assessed the persistence of immunogenicity after a single dose of ChAdOx1 nCoV-19 (AZD1222), immunity after an extended interval (44–45 weeks) between the first and second dose, and response to a third dose as a booster given 28–38 weeks after the second dose.

Methods In this substudy, volunteers aged 18–55 years who were enrolled in the phase 1/2 (COV001) controlled trial in the UK and had received either a single dose or two doses of 5×10^{10} viral particles were invited back for vaccination. Here we report the reactogenicity and immunogenicity of a delayed second dose (44–45 weeks after first dose) or a third dose of the vaccine (28–38 weeks after second dose). Data from volunteers aged 18–55 years who were enrolled in either the phase 1/2 (COV001) or phase 2/3 (COV002), single-blinded, randomised controlled trials of ChAdOx1 nCoV-19 and who had previously received a single dose or two doses of 5×10^{10} viral particles are used for comparison purposes. COV001 is registered with ClinicalTrials.gov, NCT04324606, and ISRCTN, 15281137, and COV002 is registered with ClinicalTrials.gov, NCT04400838, and ISRCTN, 15281137, and both are continuing but not recruiting.

Findings Between March 11 and 21, 2021, 90 participants were enrolled in the third-dose boost substudy, of whom 80 (89%) were assessable for reactogenicity, 75 (83%) were assessable for evaluation of antibodies, and 15 (17%) were assessable for T-cells responses. The two-dose cohort comprised 321 participants who had reactogenicity data (with prime-boost interval of 8–12 weeks: 267 [83%] of 321; 15–25 weeks: 24 [7%]; or 44–45 weeks: 30 [9%]) and 261 who had immunogenicity data (interval of 8–12 weeks: 115 [44%] of 261; 15–25 weeks: 116 [44%]; and 44–45 weeks: 30 [11%]). 480 participants from the single-dose cohort were assessable for immunogenicity up to 44–45 weeks after vaccination. Antibody titres after a single dose measured approximately 320 days after vaccination remained higher than the titres measured at baseline (geometric mean titre of 66·00 ELISA units [EUs; 95% CI 47·83–91·08] *vs* 1·75 EUs [1·60–1·93]). 32 participants received a late second dose of vaccine 44–45 weeks after the first dose, of whom 30 were included in immunogenicity and reactogenicity analyses. Antibody titres were higher 28 days after vaccination in those with a longer interval between first and second dose than for those with a short interval (median total IgG titre: 923 EUs [IQR 525–1764] with an 8–12 week interval; 1860 EUs [917–4934] with a 15–25 week interval; and 3738 EUs [1824–6625] with a 44–45 week interval). Among participants who received a third dose of vaccine, antibody titres (measured in 73 [81%] participants for whom samples were available) were significantly higher 28 days after a third dose (median total IgG titre: 3746 EUs [IQR 2047–6420]) than 28 days after a second dose (median 1792 EUs [IQR 899–4634]; Wilcoxon signed rank test p=0·0043). T-cell responses were also boosted after a third dose (median response increased from 200 spot forming units [SFUs] per million peripheral blood mononuclear cells [PBMCs; IQR 127–389] immediately before the third dose to 399 SFUs per milion PBMCs [314–662] by day 28 after the third dose; Wilcoxon signed rank test p=0·012). Reactogenicity after a late second dose or a third dose was lower than reactogenicity after a first dose.

Interpretation An extended interval before the second dose of ChAdOx1 nCoV-19 leads to increased antibody titres. A third dose of ChAdOx1 nCoV-19 induces antibodies to a level that correlates with high efficacy after second dose and boosts T-cell responses.

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*Contributed equally

Jenner Institute, Nuffield Department of Medicine (A Flaxman DPhil, D Jenkin MRCP, J Aboagye BSc, S Belij-Rammerstorfer PhD, M Bittaye PhD, F Cappuccini PhD, S Davies BSc, K J Ewer PhD, P M Folegatti MD, J Fowler BA, Prof A V S Hill FRS, A M Minassian DPhil, H Sanders BSc, E Sheehan PhD, H Smith MSc, D Woods BSc, Prof S C Gilbert FMedSci, T Lambe PhD), **Oxford Vaccine Group, Department of Paediatrics** (N G Marchevsky MSc, P K Aley PhD, B Angus MD, S Bibi PhD, P Cicconi PhD, E A Clutterbuck PhD, C Dold PhD, S Kerridge MSc, Y F Mujadidi MSc, E Plested, M N Ramasamy DPhil, H Robinson DipHE, M D Snape MD, R Song MD, M Voysey DPhil, Prof Sir A J Pollard FMedSci), **and Chinese Academy of Medical Science (CAMS) Oxford Institute** (T Lambe), **University of Oxford, Oxford, UK; NIHR Oxford Biomedical Research Centre, Oxford, UK** (N G Marchevsky, S Bibi, E A Clutterbuck, C Dold, M Voysey, Prof Sir A J Pollard); **Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK** (W Dejnirattisai PhD, J Mongkolsapaya PhD, Prof G Screaton DPhil)

Correspondence to:
Dr Amy Flaxman, Jenner Institute, Nuffield Department of Medicine, University of Oxford, Headington, Oxford, OX3 7DQ, UK
amy.flaxman@ndm.ox.ac.uk

Biomedical Research Centre, Chinese Academy of Medical Sciences Innovation Fund for Medical Science, Thames Valley and South Midlands NIHR Clinical Research Network, AstraZeneca, and Wellcome.

Introduction

The COVID-19 pandemic continues to put a substantial burden on health-care systems and a massive global effort is underway to protect populations through vaccination. COVID-19 vaccine supply shortages in many countries are causing concern about compromised immunity as the interval between the first and second dose extends beyond 12 weeks.[1] WHO recommends that the second dose of the ChAdOx1 nCoV-19 vaccine is given 8–12 weeks after the first dose because the clinical trial data provide support for good levels of protection with this interval;[2,3] however, many countries cannot obtain sufficient supplies to allow second doses to be administered by 12 weeks. These supply shortages are leading to longer intervals and uncertainty among policy makers about whether protection against COVID-19[2,3] will be maintained because no data exist on the efficacy of the immunisation schedules with intervals between the first and second dose that extend beyond this limit.

For more on **COVAX** see https://www.who.int/initiatives/act-accelerator/covax

Conversely, some high-income countries with highly vaccinated populations are considering administration of a third dose of a COVID-19 vaccine because of uncertainty about duration of immunity after the first two doses and the possible risk of breakthrough infection as new variants emerge.

ChAdOx1 nCoV-19 (AZD1222), a replication deficient adenoviral vectored vaccine that encodes the SARS-CoV-2 spike protein, is one of the most widely used vaccines globally. More than half a billion doses have been distributed to more than 168 countries across six continents, including provision through the COVAX Facility. Here, we describe tolerability and immune response to a late second dose (44–45 weeks after the first dose) of ChAdOx1 nCoV-19, and after a third dose (28–38 weeks after the second dose). We also report the persistence of antibody and cellular responses at 182 days and for antibodies up to 320 days after first dose of ChAdOx1 nCoV-19.

Methods

Study design and participants

In this substudy, we extended the data already collected as part of the UK COV001 and COV002 trials. In these trials, participants were randomly assigned to receive ChAdOx1 nCoV-19 or a meningococcal conjugate vaccine (MenACWY) as a control. Procedures, safety, immune responses, and efficacy before late vaccination and after second dose have been previously published.[2,4,5] The trials were originally planned as single-dose vaccine studies, but the strong neutralising titres seen in COV001 induced by a second dose of vaccine[4] prompted a protocol amendment to allow the addition of booster doses to most study participants across both trials. Most participants in both COV001 and COV002 were invited to receive a second dose from July, 2020, onwards. The timing of the second dose varied and allows for comparisons of immunogenicity between the recommended vaccination schedule in the UK of 8–12 weeks and a longer interval of 15–25 weeks.[3] The initial phase 1 immunogenicity group in COV001 was retained as a single-dose cohort to observe the persistence of immune responses after a single dose.

For analysis of immunogenicity after a single dose of ChAdOx1 nCoV-19, we included all participants in COV001 and COV002 who had yet to receive a second dose of vaccine and for whom immunogenicity data were available.

A substudy was added to the COV001 trial as a protocol amendment on March 1, 2021, to investigate the immunogenicity and tolerability of a third dose of the vaccine. Participants who had previously received two doses of ChAdOx1 nCoV-19 were recruited for this substudy, along with control participants, who had received two doses of MenACWY previously, to maintain blinding of reactogenicity data. All recruits received ChAdOx1 nCoV-19; for the control participants this was their first dose. For participants from this three-dose cohort to be eligible for inclusion in these analyses, they had to have an 8–16 week interval between first and second doses.

Some participants from the COV001 single-dose cohort were also offered a second dose at this time. The single dose cohort originally comprised a 1:1 ratio of ChAdOx1 nCoV-19 recipients to MenACWY controls. These participants were invited back in a 2:1 ratio, so participants who had previously received a single dose of ChAdOx1 nCoV-19 received their second dose, with an interval of 44–45 weeks, and those who had been controls received their first dose of ChAdOx1 nCoV-19. Participants were targeted for inclusion in the substudy if they had not previously been unmasked to treatment allocation or offered a vaccine as part of the UK Government COVID-19 vaccine programme. A subset of two-dose recipients (for whom reactogenicity or immunogenicity data, or both, were available) were selected for inclusion in analyses for comparison with those who received two doses 44–45 weeks apart. Participants who had a positive PCR test for SARS-CoV-2 were removed from the analysis if the infection occurred before the blood draw. Participants for this substudy were only enrolled at the Oxford site.

In the UK, the COV001 and COV002 studies were approved by the South Central Berkshire Research Ethics Committee (COV001 reference 20/SC/0145, on March 23, 2020; COV002 reference 20/SC/0179; conditional approval on April 8, 2020, and full approval on April 19, 2020). The protocol for COV001 is provided in appendix 1 and the protocol for COV002 is provided in appendix 2.

See Online for appendix 1
See Online for appendix 2

Procedures

Participants who were included as part of this substudy were vaccinated with a standard dose of ChAdOx1 nCoV-19 (5×10^{10} viral particles). For control participants who had previously received either one or two doses of MenACWY, this vaccination was their first dose of ChAdOx1 nCoV-19. For participants who had previously received ChAdOx1 nCoV-19, this vaccination was either their second dose (44–45 weeks after the first) or their third dose. These late vaccinations occurred 10 months (plus or minus 56 days) from enrolment. 7 days after vaccination participants were unmasked to treatment allocation, so that those who had received only one dose of ChAdOx1 nCoV-19 could subsequently receive a second dose, in line with national vaccination roll-out in the UK.

Participants enrolled in the substudy had blood samples taken on the day of vaccination, and then at 14 days and 28 days after vaccination to allow immunogenicity assessments to be made.

Binding antibody titres were measured using standardised single dilution total IgG ELISAs as previously described.[5] This assay was used to measure antibody responses before and after vaccination to Victoria/01/2020 SARS-CoV-2 spike protein and adapted to measure responses to beta (B.1.351) SARS-CoV-2 protein. ELISA assays to Victoria/01/2020 were performed on samples

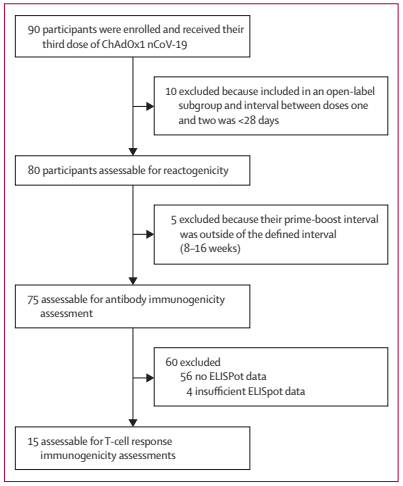

Figure 1: **Trial profile for three-dose cohort**

Research in context

Evidence before this study

Multiple vaccines against SARS-CoV-2 have now been authorised for use in various countries. Most vaccines are given in a two-dose primary schedule, and further doses might be required to maintain protective immunity or control emerging variants. We searched PubMed for research articles published between database inception and June 23, 2021, using the search terms "SARS-CoV-2", "vaccine", "clinical trial", AND ("third dose" OR "late boost") with no language restrictions. We identified animal studies using combinations of three-dose vaccine delivery in prime-boost schedules. Additionally, we identified three clinical trials of three-dose delivery, including two in solid organ transplant recipients. In the first study in transplant recipients, antibody titres increased after the third dose of either BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) vaccines in a third of patients who had negative antibody titres and in all patients who had low-positive antibody titres. In the second study in transplant recipients, prevalence of antibody titres increased from 44% after a second dose to 68% after a third dose. In a phase 1 and 2 trial of a protein subunit vaccine ZF2001, the safety and immunogenicity data support the use of a 25 μg dose in a three-dose schedule. A number of clinical studies are measuring the effect of a third dose of vaccine, including a phase 1 study of 144 participants who received a homologous third dose of BNT162b2, 6 or 12 months after the second dose.

Added value of this study

We report immune responses to ChAdOx1 nCoV-19 following a second dose after an extended interval between the first and second dose, and after a third dose with an extended interval between the second and third dose. The extended interval between the first two doses (44–45 weeks) resulted in higher antibody titres after the second dose than with a shortened interval. A third dose given 28–38 weeks after the primary series increased the antibody titres to above those after a second dose with a shortened interval. Reactogenicity was lower after the second or third dose than after the first dose.

Implications of all the available evidence

Vaccine shortages have resulted in some people receiving a first dose of ChAdOx1 nCoV-19 without receiving the second dose within the recommended 4–12 week period. We report that increasing the interval up to 45 weeks results in increased antibody titres after the second dose, offering increased flexibility in vaccination schedules. A third dose at an extended interval after the second dose resulted in a further increase in antibody titres, mitigating concerns that antibodies raised against the ChAdOx1 vector would limit repeated use of the vaccine.

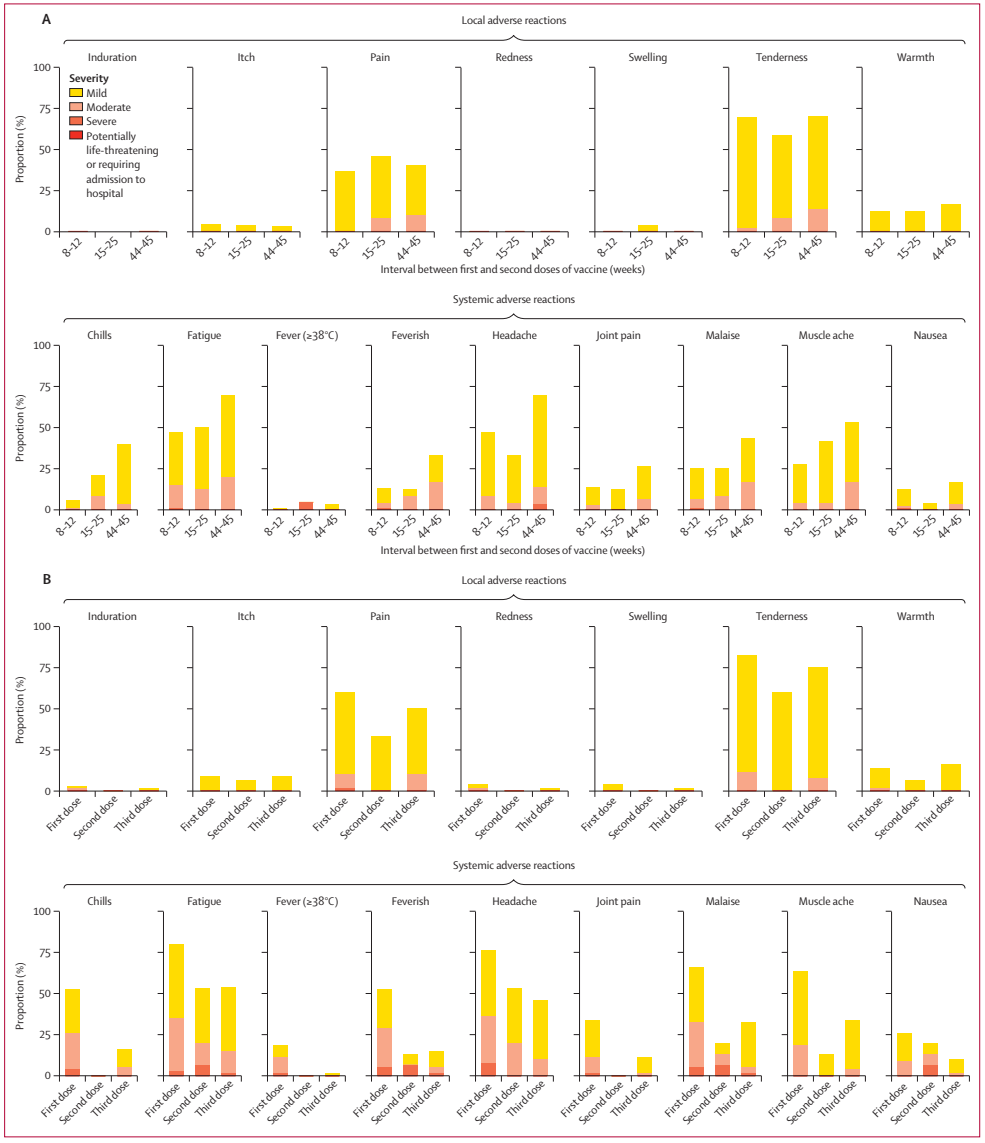

Figure 2: **Solicited adverse reactions up to 7 days after ChAdOx1 nCoV-19 vaccination by interval between first and second doses (A) and after the first, second, and third dose for participants who received a third dose of vaccine (B)**
Figure shows maximum severity of respective solicited adverse event recorded for each participant during days 0–7 after vaccination. In panel A, reactogenicity data after the second dose are shown for 263 participants for fever (≥38°C) and 267 participants for all other symptoms for the 8–12 week interval, for 23 participants for fever (≥38°C) and 24 participants for all other symptoms for the 15–25 week interval, and 28 participants for fever (≥38°C) and 30 participants for all other symptoms for the 44–45 week interval. In panel B, reactogenicity data are from after each dose recorded by participants who received a third dose of vaccine, with data available for 80 participants for all symptoms after dose 1; 15 participants for all symptoms after dose 2; and 77 participants for fever and 80 participants for all symptoms after dose 3. Participants included in panel B received their third dose 20–38 weeks after the second dose (median of 30 weeks [IQR 30–30]).

from single-dose recipients up to 1 year after vaccination, in two-dose recipients up to 6 months after the second vaccination, and in three-dose recipients up to 28 days after the third vaccination. ELISA assays on the beta SARS-CoV-2 variant were only done on samples from participants recruited to the substudy who received either a late second vaccination or a third vaccination, up to 28 days after the late vaccination. Meso Scale Discovery multiplex immunoassay was used to assess antibody titres against spike proteins from different variants (Victoria/01/2020, D614G, alpha [B.1.17], beta, and gamma [P.1]). V-PLEX SARS-CoV-2 Panel 6 (IgG) kits were used following manufacturer's instructions (Meso Scale Discovery, K15433U; full details are in appendix 3 [p 1]). Meso Scale Discovery assays were done on samples from participants recruited to the substudy who received a late second dose of vaccine (44–45 weeks after the first dose. Ex-vivo IFN-γ ELISpot assays were done as previously described[5] to assess T-cell responses to Victoria/01/2020 SARS-CoV-2 spike overlapping peptide pools before and after vaccination. Isolated peripheral blood mononuclear cells (PBMCs) were stimulated overnight with peptides spanning the SARS-CoV-2 spike insert. ELISpot assays were done on samples from participants in the single dose cohort up to 182 days after vaccination. ELISpot assays were also done in some participants (due to laboratory capacity) recruited to the substudy who received a third dose, up to 28 days after third dose. Focus reduction neutralisation assays were done as described previously[6] to measure neutralising antibody titres against alpha, beta, and delta (B.1.617.2) SARS-CoV-2 viral variants. Neutralisation assays were done in a randomly selected subset of participants (due to laboratory capacity) who received a third dose of vaccine. Timepoints assessed were 28 days after second vaccination and 28 days after third vaccination.

For all immunogenicity assessments, data were excluded upon earliest occurrence of a positive PCR test result or external COVID-19 vaccination. For single dose immunogenicity assessments, data were excluded from after receipt of second dose. For the three-dose cohort, data were included only for those who had an interval of 8–16 weeks between first and second doses.

Participants were asked to complete a diary card for 7 days after each vaccination to record solicited local (induration, itch, pain, redness, swelling, tenderness, and warmth at the injection site) and systemic (chills, fatigue, fever of ≥38°C, feverish [self-reported feeling of feverishness, whereas fever is an objective fever measurement], headache, joint pain, malaise, muscle ache, and nausea) adverse reactions. Participants reported the severity of their adverse reactions as mild, moderate, severe, or life threatening as per definitions provided (appendix 1 pp 92–94).

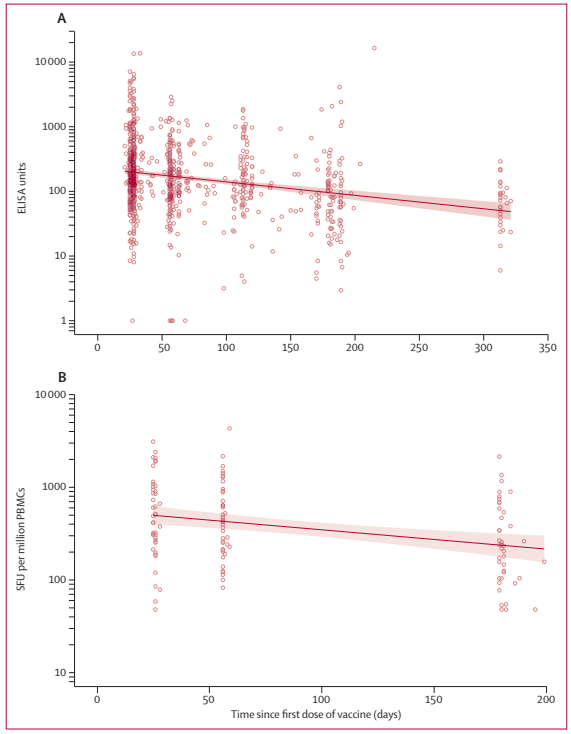

Figure 3: **Antibody (A) and T-cell (B) persistence after one dose of ChAdOx1 nCoV-19 vaccine**
Datapoints represent individual participants and the solid line represents estimates from a linear regression model, with shaded areas showing the 95% CI. Antibody levels to SARS-CoV-2 Victoria/01/2020 spike measured by total IgG ELISA over 1 year after a single dose. Data are from 480 participants across COV001 and COV002 who received a standard dose of ChAdOx1 nCoV-19. Vaccine-induced T-cell responses against the SARS-CoV-2 spike insert were monitored up to day 182 in a cohort of 44 participants who received a single dose of ChAdOx1 nCoV-19. For participants who were excluded from these analyses due to positive PCR test result, second dose on trial, or external COVID-19 vaccination, no ELISA results or ELISpot results beyond the date of censoring were used. PBMCs=peripheral blood mononuclear cells. SFUs=spot-forming units

See Online for appendix 3

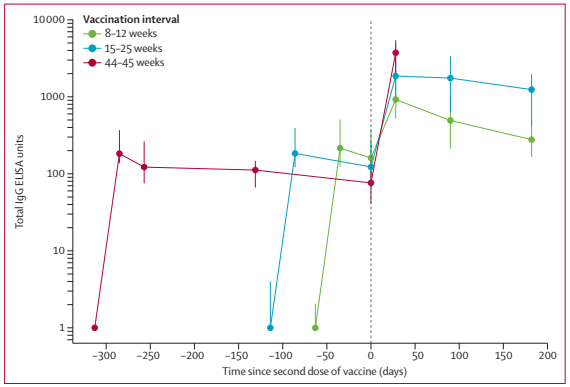

Figure 4: **Antibody response by interval between first and second vaccination**
Datapoints are medians for each group, with error bars showing IQRs. Antibody levels to SARS-CoV-2 Victoria/01/2020 spike measured by total IgG ELISA. Data are shown for 115 participants for the 8–12 week interval; 116 participants for the 15–25 week interval, and 30 participants for the 44–45 week interval. Unadjusted and age-adjusted geometric mean ratios are shown in appendix 3 (p 16).

Statistical analysis

We present summary statistics for individuals vaccinated with one, two, or three doses of ChAdOx1 nCoV-19 as median (IQR) or geometric mean titre (GMT) with 95% CIs. We do not include data from control participants (who had previously received one or two doses of MenACWY); they received ChAdOx1 nCoV-19 on recruitment to the substudy. For the purposes of ensuring trial personnel were masked to treatment assignment, data for both vaccinnees and controls were collected. Unmasking information was only available to those performing the final data analyses. Upon unmasking of participants and study personnel, control participants were excluded.

We used the Wilcoxon rank sum and Kruskal-Wallis tests for comparisons between independent groups and we used the Wilcoxon sign rank test to compare paired data. Geometric mean ratios (GMRs) with 95% CIs were produced when comparing groups. When appropriate, adjusted GMTs and GMRs were also presented to adjust for the effect of age. We did not do a sample size calculation for the immunogenicity subgroups in this analysis because of logistical considerations, including laboratory capacity. Sample size calculations for COV001 and COV002 were based on the primary efficacy outcome, which have been previously reported.

The reactogenicity cohorts included masked participants who received at least two standard doses of ChAdOx1 nCoV-19 in the two-dose cohort or three standard doses of ChAdOx1 nCoV-19 in the three-dose cohort, and had completed at least one entry in their adverse event diary after each dose. For consistency between cohorts, participants were excluded from the two-dose reactogenicity cohort if the interval between first and second dose was outside of the intervals defined in the two-dose immunogenicity cohort. All analyses of COV002 participants were restricted to those aged 18–55 years, to align with the inclusion criteria of the COV001 study, which only enrolled participants in this age range.

Our analysis of the decay of antibodies and T-cell responses over time after a single dose included all available data from timepoints up until the booster dose was administered (ie, day 28 and the day of the second dose, which varied across all participants). We modelled data using an unadjusted restricted-maximum likelihood-based mixed-effects regression approach (SAS proc mixed) with participant-level random intercepts fitted to log-transformed antibody values. We used the variance components covariance structure. We estimated GMRs and GMTs from the linear combination of model parameters. We chose the linear models after comparison with quadratic models and generalised additive (smoothed) models (GAM). The quadratic term was non-significant in the linear models and the GAM results were similar and did not substantially improve the model fits (compared using Akaike information criterion [known as AIC] statistics) from the linear models; therefore, the linear models were retained.

We did all statistical analyses using R (version 4.0.2 or later) and SAS (version 9.2). p values of less than 0·05 were considered to be significant and we made no adjustments for multiple comparisons. COV001 is registered with ClinicalTrials.gov, NCT04324606, and ISRCTN, 15281137, and COV002 is registered with ClinicalTrials.gov, NCT04400838, and ISRCTN, 15281137, and both are no longer recruiting.

Role of the funding source

AstraZeneca reviewed the manuscript before submission, but the academic authors retained editorial control. All other funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 11 and 21, 2021, 90 participants were enrolled into the COV001 third-dose boost substudy and vaccinated with ChAdOx1 nCoV-19. Ten participants from this substudy were excluded from analyses because they were enrolled from an open-label subgroup and the interval between their first and second doses was shorter than 28 days. The remaining 80 participants who were assessable for reactogenicity received their third dose of vaccine 20–38 weeks after their second dose (median 30 weeks [IQR 30–30]). A further five participants were excluded from all immunogenicity assessments because their prime-boost interval was outside the defined range of 8–16 weeks. For immunogenicity analysis of T-cell responses, another 60 participants were excluded because they did not have or had insufficient ELISpot data, leaving an analysable cohort of 15 participants (figure 1). To maintain blinding at the time of vaccination, 40 control participants (who had previously received two doses of MenACWY) were also recruited and vaccinated with ChAdOx1 nCoV-19. Data for these participants have not been included in these analyses.

Of 1110 participants from the COV001 and COV002 studies who had received a single dose of vaccine, 480 were included in the single-dose immunogenicity assessment of antibodies and 44 were included in the immunogenicity assessment of T-cell responses (appendix 3 p 2). 66 participants from the single-dose cohort of COV001 were also offered a second dose, of whom 44 were ChAdOx1 nCoV-19 recipients and 22 were control group participants who had received MenACWY. 32 (73%) of 44 participants who had previously received a single dose of ChAdOx1 nCoV-19 received their second dose, with an interval of 44–45 weeks. Two of these participants were subsequently excluded from reactogenicity and immunogenicity analyses because they had positive PCR tests for SARS-CoV-2 infection within the follow-up period, leaving 30 participants for inclusion in the two-dose cohort analyses.

The two-dose cohort comprised 321 participants from COV001 and COV002 with prime-boost intervals of 8–12 weeks (267 [83%] of 321), 15–25 weeks (24 [7%]), or 44–45 weeks (30 [9%]) who had reactogenicity data available and were included in our analyses, and 261 who had immunogenicity data available (115 [44%] of 261 had an 8–12 week interval, 116 [44%] had a 15–25 week interval, and 30 [11%] had a 44–45 week interval; appendix 3 p 3).

Baseline characteristics for the one-dose, two-dose, and three-dose cohorts are shown in appendix 3 (pp 4–5). More than 90% of participants were White. There were small differences in the median age of reactogenicity cohorts and immunogenicity cohorts. The median age of participants in the two-dose cohort antibody immunogenicity cohort with an 8–12 week interval between the first and second dose was 39 years (IQR 30–49), in the 15–25 weeks interval group was 36 years (30–43), and in the 44–45 weeks interval group was 32 years (25–44). In the three-dose cohort, the median age of participants in the reactogenicity cohort was 37 years (IQR 31–42), in the immunogenicity antibody cohort was 37 years (31–42), and in the immunogenicity T-cell response cohort 40 years (32–44).

The severity of local and systemic solicited adverse reactions 7 days after a second dose were mostly mild to moderate irrespective of the interval between doses. Local symptoms occurred after a second dose in 201 (75%) of 267 participants in the 8–12 week interval group, 15 (63%) of 24 participants in the 15–25 week interval group, and 23 (77%) of 30 participants in the 44–45 wee interval group (figure 2A; appendix 3 pp 6–9). Systemic reactions occurred in 190 (71%) of 267 participants in the 8–12 week interval group, 18 (75%) of 24 participants in the 15–25 week interval group, and 26 (87%) of 30 participants in the 44–45 week interval group (figure 2A; appendix 3 pp 6–9). 65 (81%) of 80 participants in the three-dose group reported at least one local symptom after a third dose (figure 2B; appendix 3 pp 10–14).

Second dose vaccinations in the two-dose cohort were less reactogenic than first dose vaccinations; with 72 (22%) of 321 participants reporting more than two moderate-to-severe systemic symptoms after first

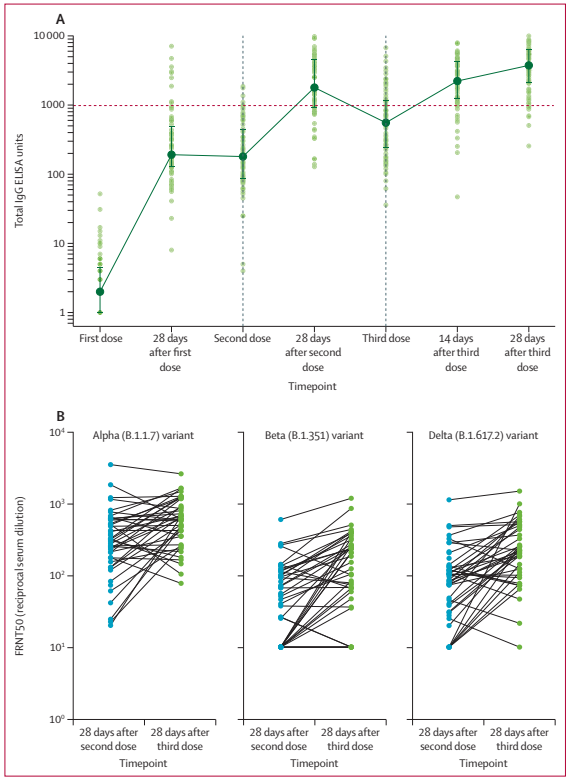

Figure 5: **Antibody responses in participants who received a third dose of ChAdOx1 nCoV-19**
(A) Antibody levels to SARS-CoV-2 Victoria/01/2020 spike protein measured by total IgG ELISA (n=75). Datapoints in lighter colours represent individual participants and darker datapoints show median values with error bars showing the IQRs and with solid lines connecting these median values. (B) Neutralisation titres from a randomly selected subset of participants (45 of 75 participants who received a third dose of vaccine and who had an interval of 8–16 weeks between their first and second dose). Datapoints represent individual participants for the three variants of concern investigated. FRNT50=focus reduction neutralisation titres with 50% neutralisation cutoff.

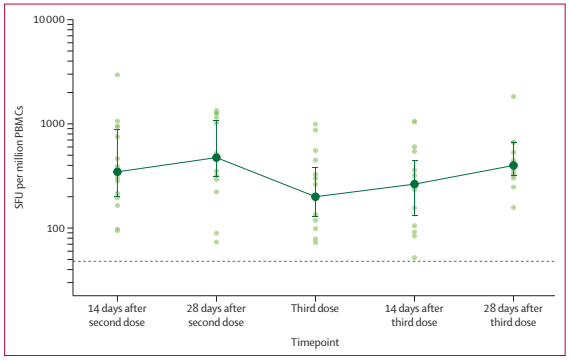

Figure 6: **IFN-γ ELISpot responses in participants who received a third dose of ChAdOx1 nCoV-19**
15 participants with an interval of 8 weeks between their first and second doses were assessed for ELISpot responses. These participants received their third dose 37–38 weeks after the second dose (median 38 weeks [IQR 38–38]). Datapoints in lighter colours represent individual participants and darker datapoints show median values with error bars showing the IQRs and with solid lines connecting these median values. The dotted horizontal line represents the lower limit of detection of the assay (48 SFU per million PBMCs). SFU=spot-forming unit. PBMC=peripheral blood mononuclear cells.

vaccination compared with 21 (7%) of 321 participants after second vaccinations (appendix 3 pp 6–9). Third dose vaccinations were also less reactogenic than first doses, with four (5%) of 80 participants in the three-dose cohort reporting more than two moderate-to-severe systemic symptoms after a third dose compared with 27 (34%) of 80 participants after the first dose (appendix 3 pp 10–14).

Antibody responses after a single dose of vaccine and measured approximately 320 days after vaccination remained higher than responses measured at baseline (GMTs of 66·00 ELISA units [EUs; 95% CI 47·83–91·08 *vs* 1·75 EUs [1·60–1·93]). At day 180, geometric mean antibody levels were half the levels observed at the day 28 peak (GMR 0·51 [95% CI 0·45–0·57]), and by day 320 were less than a third of the levels at the peak (GMR 0·27 [0·22–0·34; figure 3A; appendix 3 p 15).

Vaccine-induced cellular immune responses after a single dose of ChAdOx1 nCoV-19 followed a similar pattern of decay as antibody responses. T-cell responses decreased over the course of 6 months but were maintained above baseline levels, and at day 180 geometric mean T-cell levels were half the levels observed at the day 28 peak (GMR 0·50 [95% CI 0·41–0·60]; figure 3B; appendix 3 p 15).

Antibody levels 28 days after a second dose of vaccine were higher among those with longer intervals between doses than among those with shorter intervals between doses (median total IgG titre of 923 EUs [IQR 525–1764] for 8–12 week interval; 1860 EUs [917–4934] with 15–25 week interval; and 3738 EUs [1824–6625] with 44–45 week interval; Kruskal-Wallis test p<0·0001; figure 4; appendix 3 p 16). Age was not statistically significant in adjusted models (appendix 3 p 16).

6 months after the second dose of vaccine, antibody levels remained significantly higher in the group with a 15–25 week interval between doses compared with an 8–12 week interval (median 1240 EUs [IQR 432–2002] *vs* 278 EUs [166–499]; Wilcoxon rank sum test with continuity correction p<0·0001; figure 4; appendix 3 p 16).

IgG binding titres to all four variants tested (D614G, alpha, beta, and gamma) were significantly greater after second dose than after the first dose (p<0·0001 for all comparisons [pairwise comparisons using Wilcoxon sign rank test]; appendix 3 pp 17, 19).

Antibody responses after a third dose of vaccine were assessed in 75 participants who had received their first two doses with an interval of 8–16 weeks, and who subsequently received their third dose 28–38 weeks after the second (median 30 weeks [IQR 30–30]). Administering a third dose of vaccine boosted antibody response to Victoria/01/2020 SARS-CoV-2 spike protein (figure 5A; appendix 3 p 18). Antibody levels after the third dose were significantly higher than after the second dose (median total IgG titre was 1792 EUs [IQR 899–4634] at 28 days after the second dose *vs* 3746 EUs [2047–6420] 28 days after the third dose; pairwise comparison in 73 participants due to two samples not being available at these timepoints using Wilcoxon signed rank test p=0·0043). Binding antibody titres to the beta variant increased after a third dose (appendix 3 p 19). Neutralising antibody titres after a third dose were higher than those after the second dose against alpha (p=0·0023), beta (p<0·0001), and delta (p<0·0001) variants (Wilcoxon signed rank test; figure 5B; appendix 3 p 20).

Spike-specific cellular immune responses were measured after a third dose of ChAdOx1 nCoV-19 in 15 individuals. These individuals had received their first two doses with an interval of 8 weeks, and subsequently received their third dose 37–38 weeks after the second (median 38 weeks [IQR 38–38]). Median response increased from 200 spot-forming units (SFUs) per million PBMCs (IQR 127–389) immediately before the third dose to 264 SFUs per million PBMCs (131–452) 14 days after the third dose (p=0·57), and to 399 SFUs per million PBMCs (314–662) by 28 days after the third dose (p=0·012; figure 6; appendix 3 p 20). Peak responses at day 28 after the third dose were not significantly different to the responses after the second dose (p=0·060; appendix 3 p 20).

Discussion

Antibody levels induced by a single dose of ChAdOx1 nCoV-19 decreased gradually but remained above baseline levels after 1 year. We have previously shown that administration of a second dose of vaccine induces higher antibody responses by 1 month after the second dose than before the second dose, with higher responses with a dose interval up to 3 months between the first two doses.[3] Here, we found that a long extension of the dose interval (up to 45 weeks) between the first and second dose further enhances the immune response to the second dose when compared with shorter dose intervals. Furthermore, for the first time, we showed that a third dose of ChAdOx1 nCoV-19 can induce a strong boost to immune responses to the transgene product, SARS-CoV-2 spike protein, and that these responses result in increased neutralising antibody titres and enhanced antibody activity against variants.

The devastating impact of COVID-19 is most apparent in countries with low vaccine coverage and little healthcare infrastructure, including low-income and middle-income countries. Global vaccine shortages and policy decisions implemented at national levels have curtailed vaccine supplies for some countries where substantial numbers of individuals have already received one dose of vaccine. We have previously shown that protection against symptomatic COVID-19 is maintained after a single dose of ChAdOx1 nCoV-19 for at least 3 months, despite some waning of antibody levels[3] and we now report that the antibody levels remain above baseline for at least 1 year after single dose immunisation. These data are important for those countries where administration of a second dose is delayed because of a shortage of supply. We also showed here that an extended interval between the first and second dose of ChAdOx1 nCoV-19 results in a significantly higher antibody response 28 days after the second dose than with shorter intervals. This finding is consistent with previous data showing a longer interval between first and second dose of ChAdOx1 nCoV-19 resulted in an increase in antibody titres,[3] thus providing further reassurance that a delay in administration of the second dose will not compromise the level of protection attained. Similar findings have been reported with other vaccines;[7,8] a delayed two-dose regimen against HPV, given at least 6 months apart, results in as good or better antibody response than does three doses. A second dose of ChAdOx1 nCoV-19 is well tolerated in a delayed two-dose schedule and a third dose is also well tolerated. Reports have emerged of thrombosis and thrombocytopenia after the first dose of ChAdOx1 nCoV-19,[9] and information from Public Health England indicates that this very rare event might not occur after a second dose.[10]

If booster vaccinations against SARS-CoV-2 will be required, perhaps to counter waning immunity or to augment protection against emerging variants, is not yet known. Here, we show that a third dose of ChAdOx1 nCoV-19 is well tolerated and significantly boosts antibody titres above those measured after the second dose to the level associated with 80% vaccine efficacy, or higher, after two vaccine doses (unpublished; preprint data available[11]). Higher titre neutralising antibodies against alpha, beta, and delta variants of SARS-CoV-2 were induced 28 days after a third dose vaccination than after the second dose. Spike-specific T-cell responses were boosted after a third dose of ChAdOx1 nCoV-19 and were similar in magnitude to the responses measured after two doses. Although pre-existing immunity to human adenoviral vectors has been shown to dampen vaccine-induced immune responses,[12,13] here we found no evidence that repeated use of a replication-deficient simian adenoviral vector induces antivector immunity at a level sufficient to impair responses to further vaccination. The third dose was well tolerated by participants with lower reactogenicity than after the first dose.

Our study has several limitations, including a paucity of T-cell data after a late second dose, a paucity of tolerability data after the second dose for those who were recruited to receive a third dose, and the small number of participants who were available at 1 year after single dose who still had only received one dose (mainly due to being offered a second dose after unblinding, as per protocol). Thus far, data are only available 28 days after the third dose; however, follow-up at 6 months is planned. Participants were aged 18–55 years and caution should be taken when extrapolating our findings to beyond this age range. The sample size in this study is not sufficiently large to assess rare vaccine side-effects, but there were no tolerability concerns reported in those receiving a late second dose or a third dose booster. These results are from a mainly White population and cannot necessarily be generalised to other populations.

Here, we found that immunity induced by the viral vectored vaccine ChAdOx1 nCoV-19 is maintained for long periods after a first dose, with greater boosting of effects after the second dose after a longer interval between doses than shorter intervals. Therefore, a single dose of ChAdOx1 nCoV-19 with a second dose given after an extended period might be an effective strategy[3,14] in settings where vaccine supplies are scarce in the short term. A third dose resulted in a further increase in immune responses, including increased neutralisation of variant SARS-CoV-2 viruses, and could be used to increase vaccine efficacy against variants in susceptible populations.

Contributors
AJP, TL, MV, and DJ contributed to the writing and design of the protocol and design of the study. AJP is chief investigator. AF, JA, PKA, BA, SB-R, SB, MB, FC, PC, EAC, SD, WD, CD, KJE, PMF, JF, DJ, AMM, JM, YFM, EP, MNR, HR, HSa, ES, HSm, MDS, RS, and DW contributed to implementation of the study or laboratory experimentation, or both. AF, MV, and NGM accessed and verified the underlying study data and did the statistical analysis. TL, AF, AJP, SCG, FC, NGM, and MV contributed to the preparation of the report. TL, AF, AJP, SCG, and MV were responsible for the decision to submit the manuscript. All authors critically reviewed and approved the final version. All authors had full access to the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests
SCG and AVSH are cofounders of and shareholders in Vaccitech (collaborators in the early development of ChAdOx1 nCoV-19) and named as inventors on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and a patent application covering this SARS-CoV-2 vaccine (SCG only). TL is named as an inventor on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and was a consultant to Vaccitech. PMF is a consultant to Vaccitech. AJP is chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation, but does not participate in policy advice on coronavirus vaccines, and is a member of the WHO Strategic

Advisory Group of Experts (SAGE). AJP is an NIHR Senior Investigator. All other authors declare no competing interests.

Data sharing

The current study protocol for COV001 is provided in appendix 1 and for COV002 in appendix 2. Anonymised participant data will be made available when the trial is complete, upon requests directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

Acknowledgments

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CORRESPONDENCE

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Effects of a Prolonged Booster Interval on Neutralization of Omicron Variant

TO THE EDITOR: The coronavirus disease 2019 (Covid-19) pandemic is still ongoing,[1] and the B.1.1.529 (or omicron) variant, first detected in November 2021, has already spread globally. The ability of the omicron variant to escape vaccine-elicited immunity is of great concern. Inactivated vaccines, such as CoronaVac and BBIBP-CorV, and protein subunit vaccines, such as ZF2001, have been widely used in China and several other countries.[2]

We analyzed the binding and neutralizing antibodies elicited by three doses (two priming doses and one booster dose) of an inactivated vaccine or ZF2001, as well as those in persons who had recovered from Covid-19 (the convalescent group). The serum samples from the ZF2001 recipients were grouped according to the interval between the second and third dose; the persons in the short-interval ZF2001 group had received the second priming dose 1 month after the first dose and then the third dose 1 month after the second dose, and those in the prolonged-interval ZF2001 group had received the second priming dose 1 month after the first dose and then the third dose 4 months after the second dose. The decreases in the titers of antibodies binding to the omicron variant were greater in the serum samples from both ZF2001 groups than in those from the inactivated-vaccine group or the convalescent group (Fig. 1A through 1D and Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

We used a pseudovirus system to test the serum samples for neutralizing antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) prototype strain and variants of concern, including the omicron variant. In the convalescent group, 15 of 16 serum samples were shown to be negative for neutralizing antibodies against the omicron variant, which indicates that the immune escape potential of this variant is high — a finding consistent with those of other recent analyses.[3] However, the antibodies in the serum samples from the inactivated-vaccine and ZF2001 groups remained effective in the neutralization of the omicron variant with relatively high seroconversion. Among the persons who received three doses of either vaccine, 10 of 16 samples (62%) in the inactivated-vaccine group, 9 of 16 samples (56%) in the short-interval ZF2001 group, and 16 of 16 samples (100%) in the prolonged-interval ZF2001 group were shown to be positive for neutralizing antibodies against the omicron variant. In a fifth group of persons who also had a prolonged 4-month interval between the second and third dose of ZF2001 but whose serum samples were collected 4 to 6 months after the third dose, 9 of 13 serum samples (69%) were positive for neutralizing antibodies against the omicron variant. The titer of neutralizing antibodies against the omicron variant was lower than that against the prototype SARS-CoV-2 strain by a factor of 17.4 in the convalescent group, by a factor of 5.1 in the inactivated-vaccine group, by a factor of 10.6 in the short-interval ZF2001 group, and by a factor of 3.1 in the prolonged-interval ZF2001 group (Fig. 1F through 1J). Moreover, as we reported previously,[4] a longer interval between the second priming dose of vaccine and the booster dose appears to result in higher neutralizing antibody titers against all variants tested.

These findings support the use of multiple vaccine boosts and prolonged intervals between vaccine doses to protect against highly mutated variants such as omicron in persons who had previously received two priming doses of vaccine or who had previously recovered from SARS-

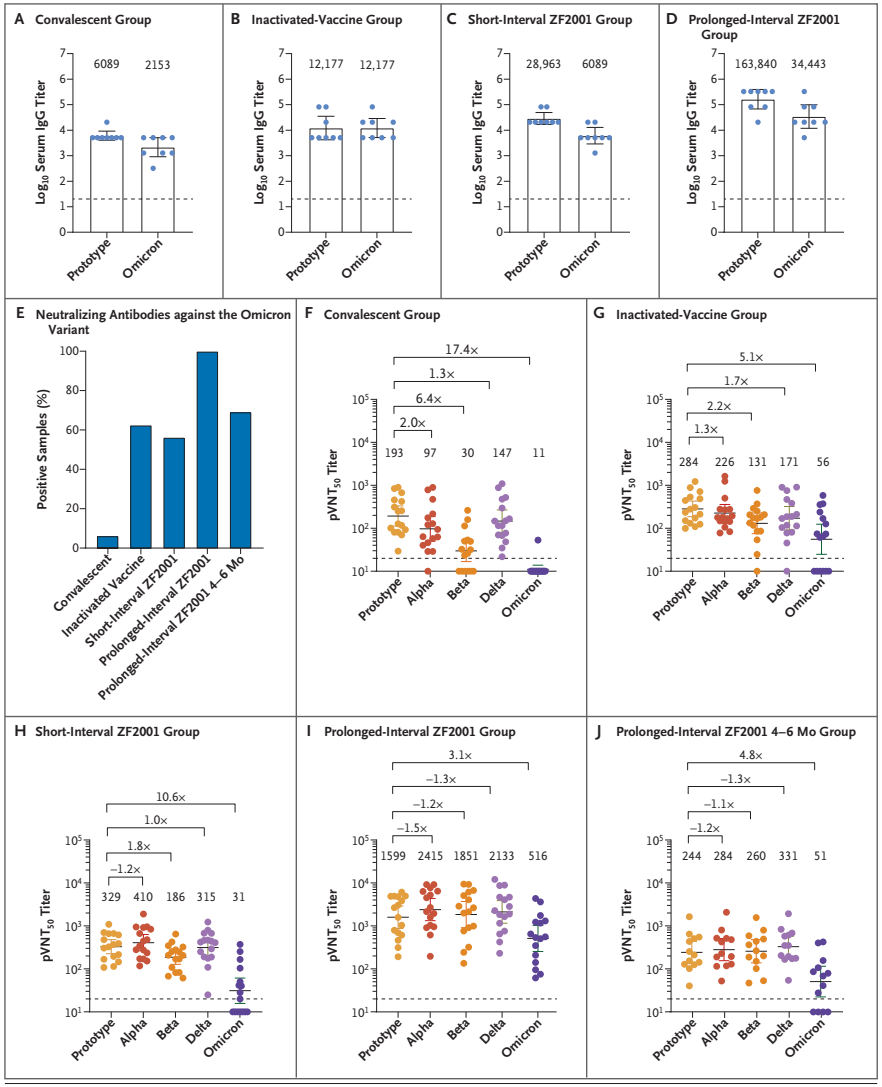

Figure 1 (facing page). Serum IgG Titers and Pseudovirus Neutralization against the Omicron Variant.

Serum samples were obtained from persons who had recovered from coronavirus disease 2019 (the convalescent group) or persons who had received three doses of an inactivated vaccine or the ZF2001 protein subunit vaccine. These samples were tested for binding and neutralizing antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) prototype and variants of concern (B.1.1.7 [or alpha], B.1.351 [or beta], B.1.617.2 [or delta], and B.1.1.529 [or omicron]). Panels A through D show the titers of serum IgG antibodies against the SARS-CoV-2 prototype strain or the omicron trimeric spike protein. The persons in the inactivated-vaccine group received the second priming dose 1 month after the first dose and then the third dose more than 6 months after the second dose. The persons in the short-interval ZF2001 group received the second priming dose 1 month after the first dose and then the third dose 1 month after the second dose. The persons in the prolonged-interval ZF2001 group received the second priming dose 1 month after the first dose and then the third dose 4 months after the second dose. A total of 8 samples from 8 persons were tested in each group. Panel E shows the percentage of samples that tested positive (as indicated by a titer of >1:20) for neutralizing antibodies against the omicron variant. "Prolonged-interval ZF2001 4–6 Mo" refers to the 13 serum samples from vaccinees who also had a prolonged interval between the second and third dose but were collected 4 to 6 months after the third dose. Panels F through I show the 50% pseudovirus neutralization titer ($pVNT_{50}$) in serum samples against the SARS-CoV-2 prototype and variants of concern; the $pVNT_{50}$ is the end-point titer of serum dilution that inhibits pseudovirus infection by 50%. A total of 16 samples from 16 persons were tested in each group. Panel J shows the $pVNT_{50}$ in the 13 samples from the prolonged-interval ZF2001 4–6 group. All neutralization assays were repeated twice. In all the panels except Panel E, geometric mean titers (GMTs) with 95% confidence intervals are shown, and the dashed lines indicate the lower limit of detection. In Panels A through D, the values above the bars are the GMT of the end-point titer in the enzyme-linked immunosorbent assay of SARS-CoV-2–binding IgG (see the Supplementary Analysis). In Panels F through J, the values above the bars are the GMT of the $pVNT_{50}$; $pVNT_{50}$ titers lower than 1:20 were considered to indicate that the sample was negative for neutralization antibodies.

CoV-2. Our results are in accordance with those of previous studies involving messenger RNA vaccine recipients.[5] Next-generation vaccines that stimulate broad protection against SARS-CoV-2 variants are also needed.

Xin Zhao, Ph.D.
Dedong Li, M.S.A.
Wenjing Ruan, B.Sc.
Chinese Academy of Sciences
Beijing, China

Zhihai Chen, M.M.
Beijing Ditan Hospital
Beijing, China

Rong Zhang, B.Sc.
Anqi Zheng, B.Eng.
Shitong Qiao, B.S.A.
Xinlei Zheng
Chinese Academy of Sciences
Beijing, China

Yingze Zhao, M.D.
Chinese Center for Disease Control and Prevention
Beijing, China

Lianpan Dai, Ph.D.
Chinese Academy of Sciences
Beijing, China

Pengcheng Han, Ph.D.
Southeast University
Nanjing, China

George F. Gao, D.Phil.
Chinese Academy of Sciences
Beijing, China
gaof@im.ac.cn

Dr. X. Zhao, Mr. Li, Ms. Ruan, and Mr. Chen contributed equally to this letter.

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