INTRODUCTION

Clinical trials of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines have collectively involved thousands of participants, providing evidence to support expedient and widespread vaccination globally (1–4). In the United Kingdom, the first vaccine to be given emergency authorization was the Pfizer-BioNTech stabilized spike-based mRNA vaccine, now marketed as BNT162b2. The vaccines developed to this point are proving to be effective in reducing the infection rate and morbidity and mortality associated with SARS-CoV-2 infection (2, 5, 6). However, there are concerns about the impact of antigenic viral variants that have recently emerged with spike protein variations, presumably in the face of sub-optimal immunity in areas of high virus circulation (7). One of these variants, first reported in South Africa (8), and known as 501Y.V2 (lineage B.1.351) or the β variant, has been shown to effectively escape from neutralization by convalescent serum samples, monoclonal antibodies, and vaccine-elicited serum samples (9–12), and poor neutralization may lead to vaccine breakthrough (13). Given the potential for antigenic variation to lead to vaccine escape, consideration is being given to provide additional boosts, either with the vaccines containing the original lineage A (Wuhan) viral spike, or with antigens matched to emerging...
variants (14). Although increased antigenic exposure is expected to elevate antibody titer and potentially expand the epitope repertoire, previous studies of other respiratory viruses, for example influenza A, have shown limits to the extent to which repeated antigenic exposure can boost antibody concentrations (15).

To investigate the potential for increased antigenic exposure to produce antibody-mediated immunity with increased neutralizing potency against SARS-CoV-2 variants, we analyzed serum samples obtained from a prospective, longitudinal cohort of healthcare workers collected at regular intervals through the period of pandemic. We analyzed serum samples collected after first and second vaccine doses from individuals previously infected with SARS-CoV-2 and seronegative individuals for the presence of spike reactive or virus-neutralizing antibodies against lineage A and lineage B.1.351 virus. We also performed neutralization assays against P.1 (v) variant pseudotypes using available post-boost serum samples.

RESULTS

Repeated SARS-CoV-2 antigenic exposure, through vaccination or natural infection, results in increased and more broadly reactive spike-specific antibody responses.

The PANTHER vaccine cohort consists of frontline healthcare workers, of which 18% had serological or polymerase chain reaction (PCR)-confirmed past SARS-CoV-2 infection prior to vaccination (16, 17). Due to occupational risk, and to reduce nosocomial transmission in hospitals, a subset of these people was included in the first-priority group for COVID-19 vaccination. For this study, we identified a total of 45 individuals who had received both doses of the BNT162b2 vaccine with complete serology and prioritized for vaccination and divided them into two groups: those with past infection and those with no evidence of past infection (table S1). The two groups did not differ in terms of age or body mass index (fig. S1), although more females (n=36) than males (n=9) were recruited into the study cohort. Before assessing the S1-specific antibody response we validated our in-house S1-specific antibody assay against the commercially available assay (Roche) and showed good correlation between the two assays (fig. S2). We then went on to measure the S1-specific antibody response, using the validated in-house enzyme immunoassay, in serum samples obtained two days before and then at least two weeks after the second (booster) vaccine dose. Following the first vaccine dose, concentrations of antibodies specific to to the lineage A (fig. 1A) and lineage B.1 (fig. 1B) S1 proteins were significantly higher in individuals with evidence of prior infection compared to those who were uninfected (median absorbance ratio of 2.43 versus 0.83; p=0.0008 and 12.72 versus 1.65; p=0.0045 respectively). Antibody concentrations in individuals with past infection and a single dose of vaccine were comparable to uninfected individuals following two doses of vaccine. The booster dose significantly increased both lineage A-specific and lineage B1-specific antibody concentrations in both groups (Lineage A, no infection 2 dose versus 1 dose, p=0.0001; B1, no infection 2 dose versus 1 dose, p<0.001). While most individuals showed an increase in S1 antibody concentrations to all variant S1 proteins, before and after plots showed that, in a small proportion (5/45 or 11%) of individuals, the antibody concentration decreased after boosting (fig. S3). One individual who had no prior exposure to SARS-CoV-2 generated a potent antibody response from a low baseline value following a single dose of vaccine comparable to that seen in individuals with prior infection. The before and after plots also showed that antibody reactivity before and after boost to the S2 subunit of spike was relatively low, when compared to S1, in most serum samples (median absorbance ratio of 1.157 versus 1.779 post boost), although did show a significant increase in the individuals that had not had a natural infection (p=0.01205). As expected reactivity to nucleocapsid (0.815 versus 0.787 post boost) was also low (fig. S3).

A similar analysis against S1 protein representing the neutralization-resistant B.1.351 S1 protein revealed more variability in recognition following multiple exposures to S1 antigen (18), although, in general, increasing antigenic exposure either through natural infection or vaccination resulted in increased reactivity. The average reactivity in uninfected individuals, following a single dose of vaccine was relatively low initially but increased significantly following the vaccine boost (median absorbance ratio of 1.24 and 5.27, respectively, p<0.0001, Fig. 1C). A single vaccine dose in previously infected people elicited significantly higher antibody reactivity (median absorbance ratio = 5.97, p=0.0006) than the prime-dose response observed in uninfected individuals. Finally, we revalidated the in-house assay against the Roche commercial spike antibody assay using samples collected post-vaccine boost, and again there was a strong correlation between the two assays (fig. S2).

Increasing antigenic exposure preferentially increases targeting of epitopes outside of the receptor binding domain.

To provide insight into the type of antibody response generated by multiple exposures, we titrated serum samples from both groups after two doses of vaccine and evaluated their reactivity against Lineage A S1 and RBD of the same strain (fig. 2A to D). The EC50 titers against the S1 region of the spike protein was significantly higher in individuals with two vaccine doses and natural infection compared to individuals who had two doses of the vaccine and no prior history of infection (6328 versus 3936; p=0.0034, Fig. 2E). The EC50 against RBD was lower than against full S1 in both cohorts, but no difference was observed between two doses with and
without natural infection (1568 versus 1039; n.s., Fig. 2E). These data suggest that the response elicited by multiple exposures to spike protein is being directed toward epitopes outside of the RBD.

**Prior infection and vaccine boosting increases neutralizing antibody titer.**

Having established that past infection and vaccine boosting increased SI-specific antibody concentrations, we used pseudotyped lentiviruses expressing either lineage A or B.1.351 spike protein to determine virus neutralization curves (Fig. S4) following each dose of vaccine in the two groups, then compared the resulting ID\(_{50}\) values (Fig. 3A). In the analyses using lineage A pseudoviruses, the average serum ID\(_{50}\) neutralizing titers following a single dose of vaccine were higher in those who had a past infection with SARS-CoV-2 compared to those without previous infection (535.4 versus 114.7; p=0.0008, Fig. 3A). Similarly, mean reciprocal neutralizing antibody titers were higher in uninfected individuals following two vaccine doses compared to previously infected individuals (1405 versus 535.4; p=0.0002) who had received a single dose. Vaccine boosting increased neutralizing antibody titers in both groups when compared to titers obtained following a single dose of vaccine. There was a 12-fold change in the mean reciprocal titer for uninfected individuals and a seven-fold increase for samples from previously infected individuals, and both increases were statistically significant (p<0.0001 for both).

We assessed the impact of antigenic variation using pseudoviruses bearing the lineage B.1.351 spike protein (Fig. 3A). Repeated antigen exposure resulted in an increase in neutralization of the B.1.351 variant. For example, in previously infected individuals, the mean ID\(_{50}\) neutralizing titer increased from about 1:60 after the initial dose of vaccine to 1:300 after the booster dose (p=0.0019). Neutralizing titers for serum samples were consistently greater against pseudoviruses complemented with the lineage A variant spike protein as compared to the neutralization-resistant B.1.351 spike variant.

To determine if the boosting effect of prior exposure was a general phenomenon, we determined the neutralizing potency of post-boost serum samples against pseudoviruses bearing a spike protein from a different variant of concern, the P.1 or γ variant first identified in Brazil. Comparison of the average reciprocal serum-dilution ID\(_{50}\) values showed significantly higher concentrations of neutralizing antibodies in serum samples obtained from individuals with prior SARS-CoV-2 infection compared to those with no known previous infection (3703 versus 1068, p=0.0074, Fig. 3B).

We then established the impact of antigenic variation on predicted vaccine effectiveness in the entire cohort by measuring pseudotype neutralization using a fixed 1:200 dilution of serum. We selected a 1:200 dilution because previous SARS-CoV-2 challenge studies in non-human primates have shown that serum samples yielding an in vitro IC\(_{50}\) neutralization titer of at least 1:200 are protective (19). Following a single dose of BNT162b2, the degree of neutralization in individuals without previous infection was significantly higher against the lineage A spike virus as compared to B.1.351 (40.9% versus 20.2%; p<0.0001). However, the average values were all less than 50% neutralization (Fig. 3C; Table S2). Neutralizing antibodies against both variants increased with increasing antigenic exposure either through natural infection or vaccination. After the final vaccine boost, the ability of serum samples to neutralize virus was significantly lower for the B.1.351 variant compared to Lineage A (p=0.0345) and P.1 (p=0.0302). However, no difference was observed after vaccine boost in previously infected individuals, with the average neutralization (Lineage A 91.6% and B.1.351 84.7%) greater than 50% for both variants with all achieving effective neutralization against Lineage A and 85% against B.1.351. Similarly, the average percentage neutralization observed for a fixed dilution of post-boost serum samples against the P.1 variant was 82% and 49% in previously infected versus SARS-CoV-2 naïve individuals, respectively (p<0.0001) (Fig. 3C; Table S2). Finally, we observed a positive correlation between total SI antibody concentrations and neutralization titers (Lineage A: r=0.5583, p<0.0001; B.1.351: r=0.4452, p<0.0001) (Fig. S5).

**DISCUSSION**

Using a well characterized healthcare worker cohort, we have been able to quantify the effect of a booster vaccination strategy on neutralizing or total IgG antibody responses to SARS-CoV-2 and variants of concern. We focused on IgG antibody concentrations as these are the predominant antibody class induced by intramuscular immunization and are associated with long-term protective immunity (20). We measured antibody responses in serum samples isolated from naïve individuals and individuals with prior history of SARS-CoV-2 infection at baseline and post-vaccination with first and second doses. Although previous studies have shown that antibody responses in previously exposed individuals are greater following a single dose of a COVID-19 vaccine (21–23), this study shows that the trend toward higher neutralizing antibody frequency in previously infected people continues after the second dose. Our results demonstrate that repeated antigenic exposure increase antibody and neutralizing responses to Lineage A, B.1, B.1.351 and P.1 variants, suggesting that vaccination with boosters may be effective in combating variants of concern.

Vaccine effectiveness against neutralization-resistant SARS-CoV-2 variants, such as the B.1.351 variant, is of major public health concern (10, 24) (also reviewed in (25)). This study confirms the neutralization-resistant phenotype of the B.1.351 variant. Following one dose of vaccine in individuals without prior SARS-CoV-2 infection, the ability of serum samples to neutralize virus was significantly lower for the B.1.351 variant compared to Lineage A (p=0.0345) and P.1 (p=0.0302). However, no difference was observed after vaccine boost in previously infected individuals, with the average neutralization (Lineage A 91.6% and B.1.351 84.7%) greater than 50% for both variants with all achieving effective neutralization against Lineage A and 85% against B.1.351. Similarly, the average percentage neutralization observed for a fixed dilution of post-boost serum samples against the P.1 variant was 82% and 49% in previously infected versus SARS-CoV-2 naïve individuals, respectively (p<0.0001) (Fig. 3C; Table S2). Finally, we observed a positive correlation between total SI antibody concentrations and neutralization titers (Lineage A: r=0.5583, p<0.0001; B.1.351: r=0.4452, p<0.0001) (Fig. S5).
with or without prior infection, spike-protein specific IgG and neutralizing antibody concentrations were lower against the B.1.351 variant than lineage A strains. However, increasing antigenic exposure in humans through natural infection followed by a two-dose BNT162b2 vaccination schedule increased IgG concentration and neutralization of the B.1.351, as well as P.1, variants to values comparable to the lineage A virus. Further characterization of the IgG response suggested that multiple antigenic exposure increases reactivity to epitopes outside of the RBD. This might contribute to the increased cross-reactivity of the antibody response and increased neutralizing activity. However, the relative contribution of repertoire expansion versus increased antibody affinity to increased antibody reactivity is unclear. Reactivity to the S2 region after vaccine boost showed only modest increases compared to S1, especially in individuals without prior exposure to SARS-CoV-2. Previous analysis of BNT162b2 antibody responses have shown that this vaccine elicits measurable S2-specific responses (26). Thus, it is unclear why robust S2 responses were not evident in all of our vaccine recipients. Future characterization of S1 and S2 neutralizing determinants, together with analysis of antibody affinity and avidity, will provide a better understanding of protective antibody responses and provide important insights for future vaccine development.

Recent studies have evaluated the durability of anti-SARS-CoV-2 neutralizing antibody titers through longitudinal sampling and modelling of phase 1 and 2 clinical data to estimate thresholds for protection against infection and against severe COVID-19. Independent of these specific thresholds, a lower degree of initial neutralization may result in a shorter time span before the titers are below the protective threshold. In the case of a 108-day half-life for titers, a 25% lower neutralizing titer for a given variant would mean 44.8 fewer days above a protective threshold, which may be relevant in terms of public health in periods when viral transmission is high.

To evaluate virus neutralization, we utilized a lentivirus-based pseudovirus assay, rather than live virus assays. This was chosen because of the superior tractability of the pseudotype assay compared to live virus neutralization assays. These assays are also less susceptible to cell culture adaptation that is frequently encountered during virus passage. In direct comparisons the pseudotype assay was shown to produce data that was entirely comparable to more laborious live virus assays (27).

Our study has several limitations. Prior exposure in our cohort involves only exposure to the lineage A or B lineage variants (28, 29), and natural infection by more antigenically diverse strains may skew the resultant immune potency and breadth. Unfortunately, we had too few positive PCR samples to try to elucidate any link between immunity and infecting lineage. Up to the start of the vaccination program, Nottingham and the surrounding region recorded a range of B.1-derived sub-lineages, with expansion of B.1.1.7 (α variant) occurring from January 2021. We did not include B.1.1.7 in our analysis as previous studies had shown that neutralizing antibody responses and vaccine effectiveness to this variant were similar to lineage A (30, 31). Instead, we focused on major variants of concern that had previously been shown to be resistant to neutralizing antibody responses (B.1.351 and P.1). However, other variants, such as the recently described B.1.617.2 (δ variant first recorded India) might respond differently. Therefore, it will be important to further validate the effectiveness of vaccine-elicited serum on a variety of emerging variants of concern. For the antibody binding analysis, we relied on the use of recombinant protein from a commercial source that did not have the P.1 variant available. To ensure data comparability we did not attempt to source the P.1 variant from an alternative supplier, or to produce it in-house. A further limitation is that we have not been able to address the links between neutralization and T cell or B cell responses. Our analyses focused on predominantly female healthcare workers rather than more vulnerable older populations, and our cohort received only one of several vaccines being rolled out globally. Finally, UK vaccination policy at the time of our study dictated an extended interval between prime and boost vaccination of between 10 and 12 weeks. Therefore, it will be important to assess whether this affected immune response in comparison to the widely adopted 3 to 4 week schedule. Related to this, a recent study has indicated increased antibody responses are associated with the extended dosing interval in those aged over 80 years (32). Despite the caveats outlined above, our data provides insight into the impact of multiple antigenic exposures and its effect on increasing antibody potency and breadth, and we expect this to be recapitulated across different vaccines and across people with different vulnerabilities. Certainly, the extensive clinical trial data and ensuing data captured during vaccine roll out suggest that approved vaccines have similar performance and that the response in different groups of recipients is also similar (4, 11, 33, 34).

Previous studies have shown that a single dose vaccine can improve antibody titers and neutralizing potency in previously infected individuals (35–39). However, this study further explores the effects of prior infection on cross-reactive immunity induced by a complete, two-dose COVID-19 vaccine schedule with administration of the first and second doses separated by 10 weeks. Our results, therefore, capture the effect of a booster dose on neutralization after antibody titers have started to wane (around 30% assuming that they peak at two weeks and have a half-life of 108 days (40)). Our data provide compelling evidence that neutralizing potency and breadth is further increased following a vaccine boost, and
this effect is even greater if the vaccine recipient has had prior infection with SARS-CoV-2. This indicates that repeated antigenic exposure improves immunity, and, in our cohort, there was no evidence for an apparent antibody ceiling, as has been reported previously for influenza A (15). This supports the notion that vaccine effectiveness, even against emerging neutralization-resistant variants, may be improved following an additional vaccine boost, even if that vaccine boost contained the original lineage A spike protein.

MATERIALS AND METHODS

Study design

The Pandemic Tracking of Healthcare workers (PANTHER) study is a longitudinal cohort study launched in April 2020 to follow up seropositivity to SARS-CoV-2 among healthcare workers (HCW) in Nottingham for 10 weeks. Further serological follow-up was performed in autumn 2020. The cohort is described in detail in the online published protocol (17) and in (16).

In January 2021 a subset of 45 participants from the above cohort who had already had the first dose of the BNT162b2 vaccine were enrolled and asked to give blood using a blood microsampling device ahead of their booster dose. They were then invited to attend a phlebotomy appointment between 14 and 21 days after their booster dose when a venous sample with serum collection was performed. Samples were collected and stored under a Human Tissue Authority license in the Nottingham Tissue Bank (License number: 11035). The study protocol was approved by Northwest – Greater Manchester South Research Ethics Committee (reference 20/NW/0395). The objective of the present study was to assess differences in serological responses post-vaccination among previously infected and infection naïve individuals.

Participants who had taken part in the previous serological surveillance study were invited to take part in the post vaccination study and the first 20 previously infected with SARS-CoV-2 (as shown from their seropositivity status during 2020) and 25 individuals without prior infection were included in the present study. No randomization was performed. Samples were blinded for experiments and unblinded at the point of data assessment. All assays were performed in duplicate (serology) or triplicate (neutralization).

The sample size of n=45 pre-boost versus post-boost vaccination was powered (80% power with p<0.05) to detect differences of 0.43 standard deviations in antibody or neutralization titers between first and second vaccination doses For the comparison between infection-naïve and previously infected individuals, the study was powered to detect (80% power p<0.05) differences of 0.87 standard deviations (SDs) between groups, which was considered sufficient given that differences of over one order in magnitude in anti-RBD titers post first vaccination dose (at least 2 SDs) between infection naïve and pre-infected individuals have been reported in a similar setting (41).

Patient samples

We have leveraged the Panther study (ISCTRIN: 15677965 Ethics Approved 27/10/2020 North West - Greater Manchester South Research Ethics Committee CPMS 47035, IRAS 285436), a longitudinal prospective observational study of health-care workers (HCW) in the East Midlands who underwent weekly quantitative serology testing from 20 April 2020 to 10 July 2020. This testing included quantitative measurements of IgG, IgM and IgA, neutralization assays, and symptomatology (17). A pre-defined nested sub-study to assess the effect of booster dose on antibody reactivity or neutralization to lineage A and two variants (B.1 and B.1.351) was carried out using samples isolated from 45 HCW (36 women and 9 men, aged 47.5 years (SD=11.4) from the Panther cohort who had paired pre-and post-booster dose serology; 20 of whom had confirmed SARS-CoV-2 infection prior to the start of vaccination. Of those with previous SARS-CoV-2 exposure, eight were diagnosed by PCR whereas the rest were identified by serological testing in either the second (12 individuals), third (4 individuals) or final quarters (4 individuals) of 2020 (table S1). All participants had two doses of the BNT162b2 vaccine between December 2020 and February 2021 and a booster dose between late February and mid-March 2021. The booster immunization was administered between 10 and 12 weeks following the first vaccine, in accordance with current UK vaccine policy (which differs from the schedule of 3 to 4 weeks adopted in other countries). Baseline serum samples were taken and analyzed before the vaccine program commenced. Then, further samples taken and analyzed on average 68 days after first vaccine dose (and 2 days before the boost) and 18 to 19 days after the boost for both groups of volunteers (table S1).

Spike-specific enzyme immunoassay

His-tagged, HEK293-expressed Spike S1 variants (lineage A (Wuhan), B.1 variant, and lineage A Spike S2 domain, and Baculovirus-expressed Nucleocapsid were produced by Sino-Biologicals (Stratech Scientific UK). His-tagged, HEK293-expressed recombinant B.1.351 variant stabilized S1was obtained from The Native Antigen Company. Enzyme immunoassays were carried out as described previously (42). In brief, recombinant proteins were immobilized onto microtiter plate wells and used to capture spike-specific antibodies present in indicated dilutions of human serum samples. Captured IgG was detected using a human IgG-specific horseradish peroxidase-conjugated antibody followed by the addition of Ultra-3,3′,5,5′-Tetramethylbenzidine (TMB) substrate (Thermo Fisher Scientific). Absorbance at 450nm was read using a GlowMax Discover spectrophotometer (Promega). Data were presented as the ratio of the absorbance (OD450nm) for the sample versus the mean absorbance of...
three separate pools (n=21) of negative control serum samples.

**Pseudovirus Neutralization Assay**

Pseudovirus neutralization assays were performed as previously described (43). Briefly, 1.5 × 10⁵ HEK293T cells were seeded overnight in 8 mL complete DMEM (Dulbecco’s Modified Eagle’s Medium supplemented with 10% fetal bovine serum (FBS) and 1% non-essential amino acids (NEAA)) in a 10 cm diameter Primaria-coated dish (Corning). Transfections were performed with 2 μg murine leukemia virus (MLV) Gag-Pol packaging vector (pcCMV-5349), 2 μg luciferase encoding reporter plasmid (pTG126), and 2 μg of a plasmid encoding SARS-CoV-2 spike (Lineage A, B.1.351 or P.1) with 24 μL polyclonal control (empty pseudotype) was used as a negative control in all experiments. Supernatants containing pseudo-viruses were harvested at 72 hours post-transfection and filtered through 0.45 μm membranes. For neutralization assays, 2 × 10⁴ VeroE6 cells per well were plated in 100 μL complete DMEM in white 96-well tissue culture plates (Corning) and incubated overnight at 37°C. The following day, supernatants containing SARS-CoV-2 pseudoviruses were incubated for 1 hour at room temperature with heat inactivated serum samples at either a 1:200 dilution or serially diluted before adding the samples to the VeroE6 cells. VeroE6 cells were incubated with inocula for 4 hours, which was then discarded and replaced with 200 μL fresh complete DMEM. After 72 hours, media was discarded, cells lysed with cell lysis buffer (Promega), and relative luciferase activity (RLU) was measured using a FLUOstar Omega plate reader (BMG Labtech). Neutralizing activities were reported as reciprocal serum dilution corresponding to 50% inhibitory dilution (ID₅₀) values and were calculated by nonlinear regression (GraphPad Prism version 9.1.2), using lower and upper bounds (0% and 100% inhibition) as constraints to assist curve fitting. Each sample was tested in triplicate.

**Statistical Analysis**

Data are presented as scatter plots with mean and SD, unless otherwise stated in the figure legends. Data were tested for normality and lognormality with D’Agostino & Pearson tests. Serology data were analyzed using Kruskal-Wallis with Dunn’s multiple comparisons test. ID₅₀ data were log transformed and analyzed either with one-way ANOVA with Tukey’s multiple comparisons test or a two-way ANOVA with Tukey’s multiple comparisons test. Proportion neutralization data were analyzed using one-way ANOVA with Tukey’s multiple comparisons test. EC₅₀ data were analyzed using Mann Whitney two-tailed test. Data analysis was blinded. Correlation between antibody concentrations and neutralizing ID₅₀ values were assessed using a Pearson correlation coefficient. Each experiment was performed once in either duplicate (serology) or triplicate (pseudovirus neutralization). Sample sizes were n=25 for uninfected and n=20 for infected, unless otherwise stated in the figure legends. Differences in means were considered statistically significant at p < 0.05. Significance levels are indicated as: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; n.s., non-significant. Analyses were performed using GraphPad Prism 9.1.0 software.

**SUPPLEMENTARY MATERIALS**

stm.sciencemag.org/cgi/content/full/sci-transmed.abj0847/DC1 Figs. S1 to S5 Tables S1 and S2 Data file S1

**REFERENCES AND NOTES**


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We would like to thank the volunteers enrolled into the... community transmission and missed intervention opportunities for SARS-CoV-2 in the United Kingdom.}(1)

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Fig. 1. Multiple exposures increase antibody reactivity to SARS-CoV-2 variants. (A to C) The ratio of reactivity signal to control (Signal:control) of Lineage A S1 (A), B.1 S1 (B) and B.1.351 S1 (C) are shown at a 1:600 serum dilution. The number of vaccine doses and infection history are indicated. Plots show median and interquartile ranges (IQR). Differences between groups were analyzed using a Kruskal-Wallis test with Dunn’s multiple comparisons correction. **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, non-significant.
Fig. 2. Prior history of SARS-CoV-2 infection increases binding to the S1 region of Lineage A SARS-CoV-2 relative to those without prior history of infection. (A and B) Binding of antibodies to Lineage A S1 was measured in serum samples isolated from participants after two doses of vaccine without prior infection (A) or with prior infection (B). OD450, optical density at 450nm. (C and D) Binding to the Lineage A RBD was also evaluated in serum samples collected after two doses of vaccine without prior infection (C) or with prior infection (D). (E) EC50 titers are shown based on prior history of infection and binding. Due to sample constraints, the two dose with infection group n=18. The plot in (E) shows mean ± SD. Differences between groups in (E) were evaluated using a one-way ANOVA with Tukey’s multiple comparisons test. **p < 0.01; ****p < 0.0001; ns, non-significant.
Fig. 3. Neutralization potency of SARS-CoV-2-specific antibodies increases with exposure to spike protein through infection or vaccination. (A) The Log10 ID50 of serum samples specific to Lineage A or B.1.351 pseudoviruses are shown. (B) The Log10 ID50 of serum against P.1 variant pseudovirus is shown for samples collected after two doses with (n=18) and without (n=20) prior history of SARS-CoV-2 infection. Sample sizes are smaller due to sample constraints. (C) Percent neutralization of serum samples diluted 1:200 are shown for Lineage A, B.1.351, and P.1 pseudoviruses. Samples are shown by number and type of exposure. Data are presented as mean ± SD (A and C) or median and interquartile range (B) due to not passing the D’Agostino & Pearson normality test. Differences between groups in (A and C) were evaluated using a two-way ANOVA with Tukey’s multiple comparisons test. Differences between groups in (B) were evaluated using a two-tailed Mann Whitney test. *p < 0.05; **p < 0.01; ****p < 0.0001. Significance values are for panel C are shown in table S2.
Two doses of the SARS-CoV-2 BNT162b2 vaccine enhances antibody responses to variants in individuals with prior SARS-CoV-2 infection


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