SARS-CoV-2 mRNA vaccine induces robust specific and cross-reactive IgG and unequal neutralizing antibodies in naive and previously infected people

Graphical abstract

Highlights

- Healthy naive and infected subjects develop robust spike IgG after full vaccination
- After the first dose, one-third of previously infected subjects lack neutralization activity
- Neutralizing antibodies are variable in previously infected and naive subjects
- Neutralizing antibodies against Delta variant are weaker than the reference strain

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In brief

Narowski et al. investigate mRNA-vaccine-induced antibody responses in 168 healthy individuals with longitudinal specimens. After complete vaccination, both previously infected and naive individuals develop comparably robust SARS-CoV-2 spike antibodies. However, neutralizing antibody response to vaccination is variable among these individuals, supporting future tailored vaccination strategies against emerging SARS-CoV-2 variants.
Understanding vaccine-mediated protection against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is critical to overcoming the global coronavirus disease 2019 (COVID-19) pandemic. We investigate mRNA-vaccine-induced antibody responses against the reference strain, seven variants, and seasonal coronaviruses in 168 healthy individuals at three time points: before vaccination, after the first dose, and after the second dose. Following complete vaccination, both naive and previously infected individuals developed comparably robust SARS-CoV-2 spike antibodies and variable levels of cross-reactive antibodies to seasonal coronaviruses. However, the strength and frequency of SARS-CoV-2 neutralizing antibodies in naive individuals were lower than in previously infected individuals. After the first vaccine dose, one-third of previously infected individuals lacked neutralizing antibodies; this was improved to one-fifth after the second dose. In all individuals, neutralizing antibody responses against the Alpha and Delta variants were weaker than against the reference strain. Our findings support future tailored vaccination strategies against emerging SARS-CoV-2 variants as mRNA-vaccine-induced neutralizing antibodies are highly variable among individuals.
levels of HCoV cross-reactive antibodies correlate with disease severity (Wang et al., 2021a). The emergence of new, increasingly infectious and virulent SARS-CoV-2 variants is causing significant concern in global human health. Prior to the dominance of the Delta variant (B.1.617.2), the US SARS-CoV-2 Interagency Group had classified the variants circulating in the United States, including Alpha (B.1.1.7), Gamma (P.1), Beta (B.1.351), and Epsilon strains (B.1.429 and B.1.427), as variants of concern (VOC). However, with the emergence of the Delta variant (del Rio et al., 2021), and subsequently the Omicron variant, other circulating variants sustained reductions in transmission, and are now termed variants being monitored (VBM), while the Delta and Omicron variants are at present the sole VOCs in the United States. As illustrated by the Delta and Omicron variants, more infectious strains of the virus can rapidly become dominant (Mishra et al., 2021), displacing other strains with potentially different levels of virulence and host immune profiles. SARS-CoV-2 variants have accumulated key mutations, particularly in the spike protein within the NTD and the RBD, and significant concerns are developing around the efficacy of currently available treatments and vaccines. Understanding factors that underly the level of defense provided by SARS-CoV-2 vaccines against the reference WIV04 strain and circulating variants is an urgent priority. As seen with the Delta and Omicron variants, novel strains that have different virulence and vaccine susceptibility can become rapidly dominant, potentially changing the degree of protection provided by the currently available vaccines (del Rio et al., 2021; Zella et al., 2021).

We previously reported that seroprevalence among a cohort of uniformly exposed emergency department health care providers was about 5% after the first peak of the infection in Washington, DC, in the late spring of 2020 (Murakami et al., 2021). PCR-confirmed documented SARS-CoV-2 infection increased to about 11% in this cohort of 237 participants by the end of 2020, an incidence of infection in line with health care providers generally in North America (Sokal et al., 2021). Here we investigate the longitudinal antibody response at three time points to the reference Wuhan strain (WIV04); seven other variants, including the currently circulating Delta variant; and four endemic coronaviruses (HCoVs). Sample collection occurred during the spring of 2020 after the pandemic’s first peak, January of 2021 in the period immediately following the roll-out of the mRNA vaccines, and after the majority of the cohort had been fully vaccinated in early March 2021. By combining PCR and serology test results, we first determined that the previous exposure to SARS-CoV-2 irrespective of the symptoms within this cohort is about 14% before vaccination. We then evaluated SARS-CoV-2 mRNA-vaccine-induced antibody isotypes, the magnitude of spike-specific and cross-reactive antibodies, and neutralizing antibodies against the reference WIV04 strain and circulating variants.

RESULTS

Determination of SARS-CoV-2 serostatus among mRNA vaccine recipients

Between June 2020 and March 2021, we enrolled 237 health care workers in a large tertiary academic medical center (George Washington University, United States), to estimate baseline serostatus and study antibody response after doses 1 and 2 of an mRNA vaccine among previously infected and naive individuals (Figure S1 and Table S1). Overall, participants were young (median age = 30 years) and healthy (82.1% reported no chronic medical conditions). Of 237 health care workers, 161 participants received the Pfizer-BioNTech mRNA vaccine (BNT162b2), and seven participants received the Moderna mRNA-1273 vaccine. Both of these vaccines use mRNA to induce the expression of stabilized full-length SARS-CoV-2 spike protein (Angeli et al., 2021). We collected a total of 424 longitudinal samples from the 237 health care workers. This was done pre-vaccination (n = 136 collected 6 months before vaccination), after dose 1 (n = 149 collected between 6 and 28 days of vaccination [median 21 days]), and after dose 2 (n = 139 collected within 66 days of vaccination [median 54 days]). At the start of the enrollment in June 2020, seven participants were seropositive, and, between June 2020 and Dec 2020, 19 additional participants experienced symptomatic, mild SARS-CoV-2 infection, detected by RT-PCR. None of the infected health care personnel in this study experienced severe disease requiring hospitalization. To determine the SARS-CoV-2 serostatus of all the participants, we measured SARS-CoV-2 nucleocapsid antibodies in pre-vaccine and dose 1 samples and spike RBD antibodies before vaccination (Figure S1). We stratified the 237 participants as 35 seropositive and 202 as seronegative by combining RT-PCR and serology test results (Figure S1). Among the 168 mRNA vaccine recipients, 20 were previously infected and 148 were naive. Analysis of self-reported symptoms following vaccination indicated that naive individuals tended to experience fewer symptoms following both vaccine doses than previously infected individuals (Figures S2 and S3).

High levels of spike IgG over IgM and IgA antibodies after dose 1 and dose 2

In symptomatic SARS-CoV-2 infections, immunoglobulin (Ig) G, IgM, and IgA antibodies are typically developed after 9 days post symptom onset (Sette and Crotty, 2021). To understand the antibody response following the doses 1 and 2 of mRNA vaccines, we measured the SARS-CoV-2 antibody isotypes against full spike and RBD antigens (Figure 1). Among the naive, antibody levels were low up to 7 days, and IgG antibodies to full spike and RBD antigens seroconverted in >95% of the participants by day 8 (Figures 1A and 1D). The predominant response was for the IgG antibodies in both naive and previously infected participants after doses 1 and 2. Among naive individuals, IgM and IgA antibodies were higher after the first dose than the second dose (Figures 1G and 1H). In previously infected individuals, IgM levels were generally lower than in the naive individuals. However, IgA levels in the previously infected individuals were comparable with those in the naive individuals after dose 1, and were unchanged after dose 2 (Figure 1H).

Comparable spike IgG levels after dose 2 in previously infected and naive individuals

Having observed that the IgG antibodies are dominant, we compared the magnitude of IgG response after doses 1 and 2 in titration ELISA assays against full spike, RBD, and NTD antigens (Figure 2). The spike IgG antibody levels, measured by the area under the curve (AUC) in titration experiments, was
robustly boosted among the previously infected participants following dose 1, but this response varied in some individuals (Figures 2A–2C). The variation of the spike IgG antibodies after the first dose was higher in naive than in the previously infected individuals. After dose 2, IgG response to spike antigens was highly focused, and the antibody levels were comparable between naive and the previously infected individuals. The magnitude of RBD antibodies correlated well to NTD and the full spike antibodies in both groups (Figures 2D and 2E).

**Naive individuals develop weaker neutralizing antibodies than previously infected individuals**

We and others have previously shown that the levels of RBD-binding antibodies correlated to the SARS-CoV-2 neutralizing antibody titers (Premkumar et al., 2020; Wajnberg et al., 2020; Dogan et al., 2021; Freeman et al., 2021). To understand the relationship between neutralizing antibodies and spike-binding antibodies after mRNA vaccination, we measured live virus SARS-CoV-2 neutralizing titers in 37 paired samples comprising 15 previously infected and 22 naive individuals after vaccine doses 1 and 2 (Figure 3). Among previously infected and naive vaccine recipients, we observed a robust correlation between SARS-CoV-2 neutralizing antibodies and the levels of the spike RBD and NTD IgG-binding antibodies and a modest correlation with full-spike-binding IgG antibodies (Figures 3C and 3D). We also noticed a moderate to high correlation between the neutralizing antibody levels and RBD and full spike serum IgA antibodies among previously infected vaccine recipients.
Strikingly, about 85% (17 out of 20) of the naive did not develop detectable levels of neutralizing antibodies >15 days after dose 1 (Figures 3A and 3-D), and in about 50% (9 out of 19) of those naive vaccine recipients neutralizing antibodies were not detectable even after dose 2 between 20 and 66 days (Figures 3B and 3D). In contrast, 66% (10 out of 15) of previously infected individuals developed neutralizing antibodies after dose 1, and after dose 2, 80% (12 out of 15) of the previously infected had neutralizing antibodies (Figure 3C). Even though both naive and previously infected individuals developed similar levels of spike-binding antibodies after dose 2, the mean and median neutralizing antibody levels among the naive recipients were at least 10- and 4-fold weaker than the previously infected vaccine recipients (cf. Figures 3C and 3D). Overall, the relationship between spike-binding and SARS-CoV-2 neutralizing antibodies typically improved between doses 1 and 2, indicated by the increase in correlation and decreased interquartile range (IQR), and followed non-monotonic relationships, which we divided into three groups. In group 1, neutralizing antibodies were undetectable after dose 1 and remained undetectable or became detectable after dose 2. In group 2, neutralizing antibody response declined between doses 1 and 2, whereas neutralizing antibody response in group 3 was detectable after dose 1 and generally improved after dose 2. In group 1, individuals in the 50–70-year-old age range poorly developed binding and functional antibodies even after complete vaccination (Figure S4). In comparison, individuals in the 20–49-year-old age range from this group also responded poorly after the first dose, but their antibody levels relatively significantly improved after the second dose. In group 2, the decline in neutralizing antibody response between doses 1 and 2 varied from 20% to 80% among previously infected and naive participants. In group 3, the improvement in the neutralizing antibody response between doses 1 and 2 was 2-fold or less.

Neutralizing antibodies developed against WIV04 strain are weaker against other circulating variants
To understand the vaccine effectiveness against variants (Delta [B.1.617.2], Alpha [B.1.1.7], Beta [B.1.351], Gamma [P.1], Kappa [1.617.1], Zeta [P.2], Iota [B.1.526]), we analyzed and compared the neutralizing activity for the reference WIV04 strain and the variants after doses 1 and 2 among naive and previously infected individuals in a multiplex surrogate neutralization assay (Figures 4A and 4B). The multiplex surrogate neutralization assay simultaneously measured antibodies that can block the interaction between RBD and ACE2 in a panel of spike antigens from the reference WIV04 strain and the above seven novel viral variants. The percentage of the ACE2 blocking antibodies in the surrogate neutralization assay with 100× diluted sera robustly correlated to the neutralizing antibody titers obtained from the BSL-3 SARS-CoV-2 neutralization assay (Figure 4C).
previously infected vaccine recipients displayed higher spike-ACE2 blocking activity compared with the naive vaccine recipients. The magnitude of RBD-binding antibodies correlated with the spike-ACE2 blocking activity against the reference strain and followed a non-monotonic relationship across the three groups as observed for the live virus-neutralization assay (Figure 3). The spike-ACE2 blocking activity of Delta and other variants in naive and pre-exposed individuals was an average 20%–30% weaker than the ACE2 blocking activity of the mRNA-vaccine-reference strain WIV04 after dose 2 (Figures 4D and 4E). Overall, people with high RBD-binding antibodies also developed better ACE2 blocking activity and vice versa (Figures 4D and 4E).

mRNA vaccine induces higher levels of endemic HCoV cross-reactive antibodies in previously infected than in naive individuals

All participants in our cohort have been previously exposed to more than one HCoV. The development of cross-reactive antibodies to HCoVs was reported previously in hospitalized patients with severe SARS-CoV-2 symptoms (Guo et al., 2021). We, therefore, measured longitudinal antibody levels against the full spike antigens from the reference SARS-CoV-2 and the four HCoVs using a titration ELISA with the samples collected at pre-vaccination and after doses 1 and 2 from previously infected and naive individuals (Figure 5). While most vaccine recipients developed antibodies to SARS-CoV-2 as expected, we observed that some previously infected and naive individuals developed strong cross-reactive antibodies to HCoV spike antigens. The cross-reactive antibody levels were more robust after dose 1 against the β-HCoVs (OC43 and HKU-1; Figures 5B–5C, 5G–5H, and 5K) than α-HCoVs (NL63 and 229E; Figures 5D–5E, 5I–5K). Similarly, the cross-reactive antibody levels against HCoVs were more pronounced in previously infected individuals than in naive individuals, marked by a sharper rise after dose 1 followed by a noticeable decline between the first and second dose (Figure 5). Notably, the levels of cross-reactive antibodies to HCoVs was reported previously in hospitalized patients with severe SARS-CoV-2 symptoms (Guo et al., 2021). The magnitude of RBD-binding antibodies correlated with the spike-ACE2 blocking activity against the reference strain and followed a non-monotonic relationship across the three groups as observed for the live virus-neutralization assay (Figure 3). The spike-ACE2 blocking activity of Delta and other variants in naive and pre-exposed individuals was an average 20%–30% weaker than the ACE2 blocking activity of the mRNA-vaccine-reference strain WIV04 after dose 2 (Figures 4D and 4E). Overall, people with high RBD-binding antibodies also developed better ACE2 blocking activity and vice versa (Figures 4D and 4E).
induced after vaccination were highly correlated among HCoVs, indicating that the cross-reactive antibodies are most likely targeting conserved epitope(s) shared among the spike protein (Figure 5).

**DISCUSSION**

Our study on binding and neutralizing antibody response after two doses of mRNA vaccines in 168 naive and previously infected individuals provides novel insights into vaccine-induced immunity against the reference strain and variants. Similar to a few independent studies, we observed comparable levels of IgG responses to spike RBD and NTD following complete vaccination in both naive and previously infected recipients (Cho et al., 2021; Ebinger et al., 2021; Gobbi et al., 2021). However, our study demonstrates that the vaccine-induced neutralizing antibody response varies among these individuals.

We and others have previously shown that RBD antibody levels correlate well to the magnitude of the neutralizing antibodies in naive and previously infected individuals. In our study, we observed similar levels of IgG responses to spike RBD and NTD following complete vaccination in both naive and previously infected recipients. However, we observed a significant increase in the neutralizing antibody response among previously infected individuals compared to naive individuals after the second dose. This finding suggests that the previously infected individuals had a stronger immune response to the vaccine, possibly due to the presence of pre-existing antibodies.

We observed that the vaccine-induced neutralizing antibody response varied among individuals, with some showing an increase and others showing a decrease. This variability may be due to genetic factors, such as the presence of polymorphisms in the ACE2 receptor, or differences in the immune response to the vaccine.

In conclusion, our study provides valuable insights into the vaccine-induced immunity against SARS-CoV-2 and its variants. Further studies are needed to understand the factors that contribute to the variability in the immune response and to develop strategies to improve vaccine efficacy in different population groups.
Markmann et al., 2021) with binding anti-RBD IgG titers over 1:160, 95% developed live virus neutralization. By contrast, after full vaccination about two-fifths of the naive and one-fifth of the previously infected vaccine recipients in our cohort—even though mean binding anti-RBD titers were significantly higher than 1:160—did not develop detectable levels of neutralizing anti-bodies in a live virus-neutralization assay. These observations suggest that antibodies developed after mRNA vaccination and natural SARS-CoV-2 infection differ. Recently, Greaney et al. (2021b) reported that mRNA-1273 elicited RBD-focused antibody response that targeted a broader range of epitopes than those produced by natural infection, suggesting one possible basis for the observed difference. What remains unexplained is the disparity in neutralizing antibodies between the previously infected in our cohort and those observed in convalescent plasma donors. One difference between these two groups is that the previously infected from our cohort also received two doses of an mRNA vaccine, while the data reported in the convalescent plasma group were collected before any vaccines were widely available. At present, the significance of this remains unclear.

Threshold levels of neutralizing antibodies for SARS-CoV-2 immunity have yet to be established, and even relatively low levels of neutralizing antibodies have been associated with protection in nonhuman primate models (Gao et al., 2020). Nonetheless, the lack of detectable levels of neutralizing antibodies in 40% of naive participants is concerning and differs from the previous reports (Ebinger et al., 2021; Naaber et al., 2021; Terpos et al., 2021). The difference between our results and other studies may indicate the importance of assay harmonization and validation of surrogate neutralization titers with authentic live virus-neutralization assays. Ebinger et al. (2021) have utilized a particular threshold of IgG level and percentage ACE2-binding inhibition activity as a surrogate for a plaque reduction neutralization test and reported that previously infected and naive individuals develop similar ACE2 blocking activities after two doses of mRNA vaccine. A study of 308 healthy individuals reported persistent neutralizing response several months after vaccination by testing the samples at low dilution in an ACE2-binding inhibition assay (Terpos et al., 2021). Nevertheless, other factors may also contribute to the differences between our observations.
and other studies, including sample collection after vaccination, amount of vaccine dose, dosing intervals, virus strain (WIV04 strain versus WIV04 strain with D614G mutation), and participant demographics. Our study utilized the WIV04 reference strain in a BSL3 live virus-neutralization assay and a multiplex ACE2-binding inhibition assay with a broader dynamic range and also evaluated the correlation between these assays within the study subjects. The samples in our study were analyzed between 1 and 4 weeks after dose 1, and between 3 and 9 weeks after dose 2 of the BNT162b2 vaccine. Naaber et al. (2021) reported significant ACE2 blocking antibodies 1 week after dose 2 (median relative optical density [OD] of 0.64 compared with 0.97 at baseline), which declined after 12 weeks (median relative OD of 0.75) among BNT162b2 vaccine recipients. A recent study involving eight subjects who received the mRNA-1273 vaccine reported persistent neutralizing antibodies 6 months after the second dose. Notably, mRNA-1273 vaccine recipients received two 100-µg doses at 28-day intervals, whereas BNT162b2 vaccine recipients received 30-µg doses at 21-day intervals (Pegu et al., 2021).

In our study, we observed that neutralizing antibody responses to vaccination fell into one of three groups. Group 1 showed weak neutralizing antibody development after the first vaccine dose and, in the aggregate, an inadequate neutralizing Ab response after complete vaccination. Group 2 showed an initial strong and protective neutralizing antibody response followed by a decline after the second dose. Group 3 showed a modest initial response followed by increased and generally protective levels of neutralizing antibody after the second vaccine dose. Understanding any biological basis underlying the different vaccination responses in these groups could broaden our understanding of those at risk for infection despite vaccination. As reported elsewhere (Collier et al., 2021), age appears to be negatively correlated with binding and neutralizing antibody development in our cohort (Figure S4). There may be factors other than age that are important to this phenomenon.

Of particular interest is group 2 with the highest level of neutralizing antibody after the first vaccine dose, subsequently declining to below that of group 3 after the second dose. The experience of this group explains to some extent the lower-than-expected levels of neutralizing antibody after full vaccination in our cohort. It does not seem likely that protective vaccine-mediated immunity, having been established in group 2 after dose 1, became inadequate after dose 2, even though measured levels of neutralizing antibodies declined. That being said, evidence of decreasing neutralizing antibody between the first and second vaccine doses has also been observed in previous reports (Cho et al., 2021), indicating a decline of transient antibody levels and/or inadequate dosing intervals. Recent studies have shown that extending the dosing interval to between 6 and 12 weeks resulted in higher levels of neutralizing antibodies after dose 2 compared with conventional 3- to 4-week regimen (Payne et al., 2021). If the pattern of vaccine response observed in group 2 in our cohort is reflected in larger populations, extending the dosing interval may be considered for this group.

Multiple reports have also surfaced recently that previously infected individuals may not need or benefit from a second dose of an mRNA vaccine (Gobbi et al., 2021; Mazzoni et al., 2021; Padoan et al., 2021; Perez Marc et al., 2021; Vicenti et al., 2021; Wise, 2021). Our data, by contrast, show that one-third of previously infected individuals did not produce detectable levels of neutralizing antibodies after the first dose (Figure 3C, group 1); after the second dose, however, 40% of these non-responders produced neutralizing antibodies. A lack of increased spike-binding antibody level after the second dose in those previously infected with SARS-CoV-2 has also been cited as evidence that the second dose is unnecessary (Gobbi et al., 2021; Goel et al., 2021; Zipeto et al., 2021). That being said, and while data are scanty, clinical evidence does not yet exist that vaccination of the previously infected confers more immunity to reinfection than does vaccination of naive recipients to primary infection (Cho et al., 2021; Teran et al., 2021). Moreover, while elevated levels of neutralizing antibodies are considered highly protective (Khoury et al., 2021), some reports have questioned this relationship (Bradley et al., 2021), and suggested that other biomarkers may better predict SARS-CoV-2 immunity (Alter et al., 2021; Butler et al., 2021; Koch et al., 2021; Natarajan et al., 2021; Rubin, 2021). Protection following vaccination has been noted in those who have not yet produced significant neutralizing antibodies, suggesting other possible protective mechanisms. One possibility involves non-neutralizing antibodies, which mediate viral elimination through antibody Fc fragment binding to host effector cells (Beaudoin-Bussières et al., 2021). At present, though, neutralizing antibodies remains the best-known correlate of protection (Earle et al., 2021) against SARS-CoV-2 infection.

Immunity against SARS-CoV-2 variants, including the Delta variant and the newly emerging Omicron variant, is a topic of great interest. Our data demonstrated that the current mRNA-vaccine-mediated neutralizing antibodies are effective against the Alpha and the Delta variants and our observations are consistent with other reports (Abu-Raddad et al., 2021). It has been argued that “affinity maturation” can, over time, produce “broadly neutralizing antibodies” (BNABs) with the ability to provide sterilizing immunity based on high affinity for conserved epitopes among variant pathogens (Sprenger et al., 2020; Muecksch et al., 2021). Persistent exposure to pathogen antigens is thought to underlie the process of affinity maturation and the development of BNABs. It follows from this that optimized (Sprenger et al., 2020) timing of SARS-CoV-2 spike antigen exposure through booster vaccinations with the currently approved mRNA vaccines may be a route to increase resistance to SARS-CoV-2 variants.

In support of this idea, there are reports (Leier et al., 2021) of the development of adequate neutralizing antibodies against SARS-CoV-2 variants following administration of mRNA vaccines to previously infected subjects, suggesting that repeated exposure (beyond that achieved by two doses of the vaccine) may contribute to maintaining competent immunity against SARS-CoV-2 variants. However, it has also been reported that binding and neutralization of the ACE2-binding site by the two most frequently elicited human antibody families (IGHV3-53/3-66 and IGHV1-2; Figure 4C) was significantly abrogated by the mutations observed in the circulating variants (Yuan et al., 2021). One possible explanation for this apparent paradox is that an initial polyclonal response during the relatively short
period after vaccination (~2 months) is effective against circulating variants. It may be the case that, as the immune response matures in vaccinated subjects, and the polyclonal response wanes, susceptibility to the variants could widen, raising the question of whether in the future variant-focused re-vaccination with modified vaccines will become necessary.

There are concerns (Lawton, 2021; Noori et al., 2021) regarding potential problems with the effectiveness of such modified vaccines; however, whether or not the current vaccine regimen, or repeated doses of the existing vaccines, will induce BNAbs against currently circulating SARS-CoV-2 variants, remains in question. In this regard, it is worth noting that a potent cross-reactive antibody (Moyo-Gwete et al., 2021) derived from a SARS-CoV-2-variant epitope has recently been reported, suggesting that modified vaccines produced in this fashion may prove broadly effective. While, at present, vaccine-mediated immunity against the currently dominant Delta variant appears effective, constant mutation by the virus in large, unvaccinated populations against the currently dominant Delta variant appears effective, broadly effective. While, at present, vaccine-mediated immunity against the currently dominant Delta variant appears effective, constant mutation by the virus in large, unvaccinated populations in which SARS-CoV-2 transmission continues unabated suggests the potential for evolving a highly divergent variant such as Omicron against which the current vaccines may be less effective (Callaway, 2021; Krause et al., 2021; Yuan et al., 2021).

Recent studies have reported declining of SARS-CoV-2 neutralizing antibodies >6 months after receipt of the second dose of mRNA vaccine, provoking debate over mass booster vaccination (Goldberg et al., 2021). Widely administered booster immunizations against reference/variants will expose a previously vaccinated and sensitized population to risk of vaccine reaction with attendant risks and discomforts (Remmel, 2021). Our data show that previous SARS-CoV-2 exposure either from infection or vaccination results in greater symptoms in response to a second exposure to SARS-CoV-2 antigens; however, a recent small-scale study that looked at booster vaccinations with one of the two mRNA vaccines reported no more than moderate symptoms in just 15% of subjects (Wu et al., 2021).

Along with neutralizing IgG, the production of anti-spike IgA in the airways has been proposed to prevent SARS-CoV-2-reinfection (Cervia et al., 2021; Dispensieri et al., 2021). Mucosal immunity plays a vital role in viral respiratory infections; IgA dominates early immune response with development of mucosally oriented plasmablasts in natural SARS-CoV-2 infection (Sterlin et al., 2021). Neutralizing mucosal IgA has been associated with milder SARS-CoV-2, and circulating neutralizing IgG with more severe disease (Butler et al., 2021). The importance of mucosal protection in SARS-CoV-2 has led some to advocate nasally administered vaccines that induce more robust mucosal IgA responses (Wang et al., 2021b). In our study, parenterally delivered mRNA vaccines induce high levels of anti-spike IgG, similar to what is seen in severe disease. IgA production, by contrast, is induced in more modest quantities, comparable with what is seen in mild disease (Rötgen et al., 2021). Induction of strong IgG, and low IgA levels in naive patients after receiving one of the two mRNA vaccines, has also been recently reported in other independent studies (Danese et al., 2021). Serum IgA levels are not a direct measure of secretory IgA levels, since systemic IgA is predominantly made of monomeric IgA1 subclass, and mucosal IgA is a polymeric IgA2 subclass (Crago et al., 1984). However, induction of mucosal IgA after parenteral mRNA vaccination has been reported (Ketas et al., 2021). Our data show that, after full vaccination, spike IgA levels in serum correlated better to the levels of neutralizing antibodies in previously infected vaccine recipients than among naive recipients, a phenomenon that may be related to a more mature immune response in the former group and recall of memory B cells from natural infection.

In our study, naive subjects experienced significantly increased spike antibody levels against the SARS-CoV-2 reference strain, SARS-CoV-2 variants, and the HCoVs after both doses of vaccine. Previously infected subjects after dose 2, by contrast, saw modest improvement in antibodies against SARS-CoV-2, but antibody levels were stable or declining against HCoV. HCoV memory B and T cells have been implicated in the early response to SARS-CoV-2 exposure (Sokal et al., 2021) and likely explain the induction of HCoV antibodies in infection/vaccination after the first dose in both the previously infected and the naive. As has been shown with Zika and dengue viruses (Montoya et al., 2018; Premkumar et al., 2018), which are closely related flaviviruses, transient cross-reactive antibodies develop in the acute phase (Dispensieri et al., 2021) and tend to fade as immune responses mature. Nonetheless, cross-reactive HCoV antibodies targeting conserved regions were reported to be boosted in some hospitalized patients (Anderson et al., 2021; Guo et al., 2021; Song et al., 2021) and have been associated with the development of neutralizing antibodies (Dispensieri et al., 2021).

Reliable serological assays to monitor community transmission of SARS-CoV-2 and its variants, and to support post-acute SARS-CoV-2 (PASO) studies, are urgently needed. After vaccination, spike antigen serology is unsuitable for detecting SARS-CoV-2 infection; in those immunized with one of the currently available mRNA vaccines, or similar, which do not expose the recipient to the totality of viral antigens, serological assays targeting antigens not included in the vaccine may be used to identify evidence of previous infection. Currently antibodies against the nucleocapsid phosphoprotein are used for this purpose; however, our analysis of longitudinal samples shows that nucleocapsid serology displays poor sensitivity over time (Figure S1E). SARS-CoV-2 non-structural proteins such as open reading frame (ORF)-8 and ORF-3b (Alkansa et al., 2021; Barik, 2021) have been identified as alternate antibody targets, which in combination may improve sensitivity for detecting previous SARS-CoV-2 infection. These and other non-spike viral epitopes (Lorenzo et al., 2021) constitute a promising strategy to distinguish past SARS-CoV-2 infection from vaccination.

**Conclusions**

Our data show a significant lack of neutralizing antibodies in naive subjects after full vaccination, and a more limited but concerning lack of neutralizing antibodies in some previously infected individuals. In contrast to what has previously been reported, we find that previously infected individuals may benefit from two doses of the currently available mRNA vaccines. Overall, robust binding antibodies are developed after complete vaccination; however, the neutralizing antibody response to mRNA vaccination is highly variable among individuals. In general, neutralizing antibody effectiveness against the variant strains, including the Delta variant, is moderately reduced compared with the vaccine referent strain. In a subset of
individuals categorized as group 2, our data do not show improving neutralizing antibodies against circulating variants with repeated vaccine doses with the conventional 3- to 4-week regimen. Combined with the overall weak neutralizing antibody responses in group 1 after complete vaccination, this suggests that further booster vaccinations, possibly modified for the variants, may be called for in the future. We also find that nucleocapsid IgG wanes over time and is therefore limited as an assay for previous infection with SARS-CoV-2.

Limitations of the study
Study limitations include the inclusion of subjects receiving only mRNA vaccines, and variability in the time interval between vaccine administration and sample collection among participants. Our cohort’s previously SARS-CoV2-infected participants were unlikely to have been infected with one of the variants, and, to our knowledge, none of the previously infected subjects in our study could have been infected with the currently circulating Delta or Omicron variants. Study participants included primarily young, healthy adults; chronically ill, immunocompromised, and those at the extremes of age are not represented in our study. Our data represent interim values and the durability of vaccine-induced antibody response >6 months after vaccination requires further investigation. Cellular immunity is also clearly very important in the defense against infection with SARS-CoV-2; information regarding T cell responses to infection and vaccination is not included in the current paper, but is planned for future study.

STAR METHODS

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

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2022.110336.

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AUTHOR CONTRIBUTIONS

J.E.L. and L.P. developed the conceptual ideas and designed the study. K.R., J.H., and J.E.L. recruited patients and provided all clinical data. T.M.N., R.J., L.E.A., N.A.V., and L.P. performed the experiments and data analysis. A.M.d.S., R.S.B., J.E.L., and L.P. supervised the study. J.E.L. and L.P. prepared the figures and wrote the paper. A.M.d.S., R.S.B., J.E.L., and L.P. provided funding. All authors provided critical comments on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests. Subjects were drawn from the workforce of the Emergency Department at George Washington University Hospital and included over 200 health care providers. Every member of this group was invited, including all genders and ethnicities represented among eligible subjects. Anyone who expressed interest was enrolled, and no individual/group was excluded.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. One or more of the authors of this paper self-identifies as a member of the LGBTQ+ community.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Horseradish peroxidase conjugated Goat Anti-Human IgG | Jackson ImmunoResearch | 109-035-008; RRID:AB_2337579 |
| Horseradish peroxidase conjugated Goat Anti-Human IgM | Jackson ImmunoResearch | 109-035-043; RRID:AB_2337581 |
| Horseradish peroxidase conjugated Goat Anti-Human IgA | Jackson ImmunoResearch | 109-035-011; RRID:AB_2337580 |
| Mouse anti-MBP      | New England Biolabs | E8032L; RRID:AB_1559730 |
| Alkaline phosphatase-conjugated Goat Anti-Human IgG | Sigma-Aldrich | A9544; RRID:AB_258459 |
| Alkaline phosphatase-conjugated Goat Anti-Human IgM | Sigma-Aldrich | A3437; RRID:AB_258080 |
| Alkaline phosphatase-conjugated Goat Anti-Human IgA | Abcam | AB97212; RRID: AB_10695943 |
| Bacterial and virus strains |        |            |
| E.coli NEB 5-alpha F’/’q | New England Biolabs | C2992H |
| E.coli BL21(DE3)pLysS | Premkumar Lab | N/A |
| SARS-CoV-2 luciferase reporter virus | Baric lab | N/A |
| Biological samples  |        |            |
| Human sera          | This manuscript | Table S1 |
| Chemicals, peptides, and recombinant proteins |        |            |
| Halotag PEG biotin ligand | Promega | G8281 |
| HCoV-NL63 spike protein | Sino Biological | 40604-V08B |
| HCoV-OC43 spike protein | Sino Biological | 40607-V08B |
| HCoV-229E spike protein | Sino Biological | 40605-V08B |
| HCoV-HKU1 spike protein | Sino Biological | 40606-V08B |
| Strep-Tactin® Sepharose resin | IBA Lifesciences GmbH | 2-1201-002 |
| Ni-NTA Agarose      | Qiagen | 30210 |
| Nano-Glo® Luciferase Assay System | Promega | N1120 |
| Streptavidin        | Invitrogen | 434302 |
| 3,3',5,5'-Tetramethylbenzidine Liquid Substrate | Sigma-Aldrich | T0440 |
| NAP-Blocker         | G-Biosciences | 786-190 |
| p-Nitrophenyl phosphate substrate | Sigma-Aldrich | N2770 |
| Critical commercial assays |        |            |
| V-PLEX SARS-CoV-2 Panel 13 (ACE2) Kit | MESO SCALE DIAGNOSTICS, LLC | K15466U-2 |
| Experimental models: cell lines |        |            |
| Vero E6             | ATCC | CRL1586 |
| Expi293 expression system | Thermo Fisher Scientific | A14635 |
| Recombinant DNA     |        |            |
| SARS-CoV-2 nucleocapsid expression plasmid | Addgene | 157867 |
| SARS-CoV-2 full-length spike (S-2P) expression plasmid | This manuscript | N/A |
| SARS-CoV-2 full-length spike RBD expression plasmid | Premkumar et al. (2020) | GenBank: MT649401 |
| SARS-CoV-2 full-length spike NTD expression plasmid | This manuscript | N/A |
| Software and algorithms |        |            |
| Prism Version 9     | GraphPad | Version 9.3.0 (345) |
| PyMol Molecular Graphics System | Schrodinger LLC | V 2.3.4 |
**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Lakshmanane Premkumar (prem@med.unc.edu).

**Materials availability**
This study did not generate new unique reagents.

**Data and code availability**
The published article includes all datasets generated or analyzed during this study. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request. This study did not generate/analyze computer codes or algorithms.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Clinical study**
In this study, a total of 424 venous blood samples were collected from 237 unique ED HCP participants at George Washington University Hospital (GWUH) during the timeframe of June 2020 to March 2021. Clinical roles of study participants included physicians, advanced practice providers, nurses, technicians, respiratory technicians, and environmental services personnel. Samples were collected during three separate time periods. Testing occurred in May/June 2020, January of 2021, and March 2021. Participants were encouraged to participate in subsequent testing rounds so that interval serologic changes in response to SARS-CoV-2 exposures or vaccinations could be analyzed. The study was approved by the George Washington University IRB#: NCR202406.

**Patient recruitment**
Emails were sent to all GWUH ED HCP personnel through an ED staff listserv. Additionally, in order to reach those not on the listserv, notifications were sent out through GWUH’s ED nurse/technician scheduling system and fliers were placed in break rooms with QR codes connecting to patient sign-up forms. Participation days and times overlapped nurse and technician shift changes in order to encourage on-going and off-going staff to participate. All ED HCP personnel who chose to participate in this study provided written informed consent. All personnel who consented to participate were included in the study.

**Patient data collection**
All participants were asked to complete a questionnaire about demographics (age, gender, race/ethnicity, home city), ED HCP occupation, non-ED HCP affiliations (e.g. also work in the ICU), past medical history (PMH), current medications, smoking history, history of known positive COVID-19 status, recent/intercurrent viral syndrome symptoms (fever, fatigue, dry cough, anorexia, body aches, dyspnea, sputum, sore throat, diarrhea, nausea, dizziness, headache, vomiting, and abdominal pain), relative number of COVID-19 exposures at work and outside of work, and personal protective equipment (PPE) wearing habits (time spent wearing and frequency of changing surgical masks, N95 masks, and Powered Air Purifying Respirators. Additionally, participants in the second and third rounds of testing were asked questions regarding COVID-19 vaccine status, type of COVID-19 vaccine received, dates of first and second vaccine doses, and recent/intercurrent viral syndrome symptoms after obtaining COVID-19 vaccinations. We analyzed the demographic characteristics, including age and sex of the vaccine recipients (Table S1), and assessed symptomology experienced by study participants following each vaccine dose. The number (Figure S2) and types of symptoms (Figure S3) were assessed using simple descriptive statistics and then stratified by prior SARS-CoV-2 exposure (naive or pre-exposed).

**Specimen collection**
Venous blood samples were collected from each participant during each round of testing. 10 ML of blood was drawn into an SST tube, and refrigerated overnight to allow for serum separation. Subsequently the serum was drawn off into a 2 ML Eppendorf tube and stored at −80°C for subsequent use. We determined the serostatus of the vaccine recipients into previously infected and naïve (Figure S1) by combining the RT-PCR test results reported elsewhere and the serological assays described in the STAR Methods section.

**Cells**
Expi293 cells were purchased from Thermo Fisher Scientific and cultured in Expi293 expression medium in a 37°C incubator with 8% CO2 on an orbital shaker rotating at 125 rpm for spike protein expression. E.coli NEB 5-alpha F’IQ strain was used for vector construction and E.coli BL21(DE3)pLysS strain for Nucleocapsid protein expression. The bacterial strains were cultured in LB (Luria-Bertani) medium at 37°C. Vero E6 (ATCC # CRL1586) was purchased from ATCC and preserved in our laboratory.
METHOD DETAILS

Protein expression and purification
The expression and production of halo-tagged SARS-CoV-2 and the four human endemic CoV RBD antigens from mammalian cells were previously described (Premkumar et al., 2020). The halo-tagged SARS-CoV-2 NTD antigen (16–305 amino acids, Accession: P0DT2C.1) was designed and expressed in mammalian Expi293 cells as described for RBD antigens. RBD and NTD antigens were site-specifically biotinylated using Halotag PEG biotin ligand (Promega), following the manufacturer’s protocol. For producing SARS-CoV-2 spike protein trimers, a codon-optimized gene was synthesized to encode for a prefusion-stabilized SARS-CoV-2 spike protein (16–1208 amino acids, Accession: P0DT2C.1) with an N-terminal human serum albumin secretion signal peptide and C-terminal T4 foldon trimerization domain, TEV protease cleavage site, His8 tag, and the twin-strep tag. The prefusion-stabilized SARS-CoV-2 construct contains two consecutive proline substitutions in the S2 subunit as described before (Walls et al., 2020; Wrapp et al., 2020). The synthetic gene was cloned between KpnI and XhoI sites of the mammalian expression plasmid p2xH. SARS-CoV-2 spike protein was expressed as described for RBD and NTD antigens in Expi293 cells and purified from mammalian cell culture supernatant using Strep-Tactin immobilized affinity resin (IBA Lifesciences). The bacterial expression construct for full-length SARS-CoV-2 nucleocapsid was a gift from Nicolas Fawzi (Addgene plasmid # 157867; http://n2t.net/addgene:157867; RRID:Addgene:157867) (Perdikari et al., 2020). The MBP fused nucleocapsid protein was expressed and purified from BL21(DE3)PlysS using Ni-NTA affinity chromatography as described before (Premkumar et al., 2018). The purified full-length ectodomain of the human coronavirus spike proteins (HCoV-NL63, 40604-V08B; HCoV-OC43, 40607-V08B, HCoV-229E, 40605-V08B, and HCoV-HKU1, 40606-V08B) were purchased from Sino Biological.

Generation 2 RBD or NTD ELISA
All serum samples tested by ELISA assay were heat-inactivated at 56°C for 30 min to reduce risk from possible residual virus in serum. Briefly, 50 μL of Streptavidin (Invitrogen) at 4 μg/mL in Tris-Buffered Saline (TBS) pH 7.4 was coated in the 96-well, high-binding microwell assay plate (Greiner Bio-One cat # 655061) for 1 hour at 37°C. The coating solution was removed, then 100 μL of blocking solution, 1:1 Non Animal Protein-BLOCKERTM (G-Biosciences) in TBS was added for 1 hour at 37°C. Serum samples were diluted at 1:40, or serially diluted (1:100 – 1:8100), in 3% Bovine Serum Albumin (BSA) in TBS containing 0.05% Tween 20 (TBST) with biotinylated spike RBD or NTD antigen at 1 μg/mL in a 96-round-well V bottom plate (Diaogo cat # R96-300V) and incubated for 1 hour at 37°C. The blocking solution was removed, then 50 μL of diluted serum was added to the assay plate and incubated for 15 minutes at 37°C. The plate was washed three times using wash buffer (TBS containing 0.2% Tween 20), then 50 μL of horseradish peroxidase-conjugated secondary Goat Anti-Human secondary antibody at 1:40,000 dilution in 3% milk was added for 40 minutes at 37°C. For measuring total Ig, a mixture of anti-IgG, anti-IgM, and anti-IgA were added together (Jackson ImmunoResearch). For measuring isotype specific antibody, only the respective goat anti-human HRP conjugated IgG, IgM, or IgA was used. The plate was washed three times with wash buffer, and 50 μL of 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate (Sigma-Aldrich) was added to the plate, and absorbance was measured at 450 nm using a plate reader (Molecular Devices SpectraMax ABS Plus Absorbance ELISA Microplate Reader) after stopping the reaction with 50 μL of 1 N HCl.

Full-length spike protein ELISA
Briefly, 50 μL of full-length spike protein at 2 μg/mL in TBS pH 7.4 was coated in the 96-well, high-binding microwell plate (Greiner Bio-One cat # 655061) for 1 hour at 37°C. The coating solution was removed, then 100 μL of blocking solution (3% milk in TBST), was added for 1 hour at 37°C. Serum samples were diluted at 1:40, or serially diluted (1:100 – 1:8100), in the blocking solution. The blocking solution was removed, then 50 μL of diluted serum was added to the plate and incubated for 1 hour at 37°C. The plate was washed three times using wash buffer (TBS containing 0.2% Tween 20), then 50 μL of horseradish peroxidase-conjugated secondary Goat Anti-Human secondary antibody at 1:40,000 dilution in 3% milk was added for 1 hour at 37°C. For measuring isotype specific antibody, only the respective goat anti-human HRP conjugated IgG, IgM, or IgA was used (Jackson ImmunoResearch). The plate was washed three times using wash buffer, and 50 μL of 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate (Sigma-Aldrich) was added to the plate, and absorbance was measured at 450 nm using a plate reader (Molecular Devices SpectraMax ABS Plus Absorbance ELISA Microplate Reader) after stopping the reaction with 50 μL of 1 N HCl.

Live virus neutralization assay
We utilized a SARS-CoV-2 luciferase reporter virus (nLuc) to assess neutralizing antibody activity (Hou et al., 2020). Vero E6 cells were plated at 2×10⁵ cells/well in a black 96-well clear bottom plate (Corning). Heat-inactivated serum was diluted 1:20 immediately, followed by a 3-fold dilution series up to eight dilution spots in DMEM supplemented with 5% FBS. Diluted serum was incubated in a 1:1 ratio with SARS-CoV-2-nLuc to result in 75 PFU virus per well. Serum-virus complexes were incubated at 37°C with 5% CO₂ for 1 hour. Following incubation, serum-virus complexes were added to the plated Vero E6 cells and incubated for 48 hours at 37°C with 5% CO₂. After incubation, luciferase activity was measured with the Nano-Glo Luciferase Assay System (Promega) according to the manufacturer specifications. Neutralization titers (EC50) were defined as the dilution at which a 50% reduction in RLU was observed relative to the virus (no antibody) control.
Multiplex surrogate neutralization assay
A multiplexed Meso Scale Discovery (MSD) immunoassay (MSD, Rockville, MD) was used to measure the ACE2 blocking antibodies to a panel of spike proteins from SARS-CoV-2 reference strain and circulating variants, including Delta, Alpha, Beta, Gamma, Iota variants using the MSD V-PLEX SARS-CoV-2 Panel 13 (ACE2) kit according to the manufacturer’s instructions. Briefly, plates were blocked with MSD Blocker A for 30 minutes and then washed three times prior to the addition of reference standard, controls and heat-inactivated samples diluted 1:100 in diluent buffer. Plates were incubated for 1 hour with shaking at 700 rpm. A 0.25μg/ml solution of MSD SULFO-tag conjugated ACE2 was added and incubated for 1 hour with shaking, plates were washed and read with a MESO QuickPlex SQ 120 instrument. Each plate contained duplicates of a 7-point calibration curve with serial dilution of a reference standard and a blank well. Results were reported as percent inhibition calculated based on the equation ((1 – Average Sample ECL Signal / Average ECL signal of blank well) x 100).

Nucleocapsid assay
Wells of a high-binding microtiter plate (Greiner Bio-One cat # 655061) were coated with 50 μL anti-MBP (New England Biolabs) at 3 μg/mL in TBS pH 7.4, and then blocked with 100 μL of blocking solution (3% non-fat milk in TBST). The plate was washed three times using wash buffer (TBS containing 0.2% Tween 20). 50 μL of 2 μg/mL MBP fused full-length nucleocapsid protein and MBP proteins in blocking solution were added to respective wells. The plates were incubated for 1 hour at 37°C. The plates were washed 3 times, then 50 μL of heat-inactivated serum at 1:40 dilution was added to wells containing full-length N protein and MBP proteins were added respectively and further incubated for 1 hour at 37°C. The plates were washed with wash buffer, then 50 μL of alkaline phosphatase-conjugated secondary goat anti-Human anti-IgG (Sigma Cat), anti-IgA (Abcam), and anti-IgM (Sigma) at 1:2500 dilution was added and incubated for 1 hour at 37°C. The plate was washed, and 50 μL p-Nitrophenyl phosphate substrate (SIGMA FAST) was added to the plate and absorbance measured at 405 nm using a plate reader (Molecular Devices SpectraMax ABS Plus Absorbance ELISA Microplate Reader). Appropriate control sera were included in the study.

QUANTIFICATION AND STATISTICAL ANALYSIS
Characteristics of the sample were summarized using simple descriptive statistics. To describe the magnitude and spread of full spike and RBD binding IgG, IgM, and IgA titers, we generated jittered dot plots stratified by antibody isotype, time of sampling (post dose 1 and post dose 2), and prior exposure to SARS-CoV-2. Line graphs stratified by time of sampling were used to describe trends in change in antibody levels between doses. The strength of the correlations between spike RBD and spike NTD titers, between spike RBD and full Spike titers, between spike binding antibody titers and SARS-CoV-2 live virus neutralization titers, and between SARS-CoV-2 live virus neutralization titers and ACE2 blocking Ab percent, were evaluated using a two-tailed Spearman’s rank correlation in Prism 9. Neutralizing antibody responses were grouped into Group 1 (undetectable after dose 1 and remained undetectable or became detectable after dose 2), Group 2 (declined between doses 1 and 2), and Group 3 (improved between doses 1 and 2), and Spearman’s coefficient was estimated to assess correlations between neutralizing and binding antibody titers, stratified by vaccine dose. Cross-reactivity between WIV04 and three SARS-CoV-2 variants (B.1.1.7, B.1.351, and P.1), and cross-reactivity between SARS-CoV-2 and other endemic human coronaviruses was compared using stratified description of antibody titers and kinetics following each vaccine dose.