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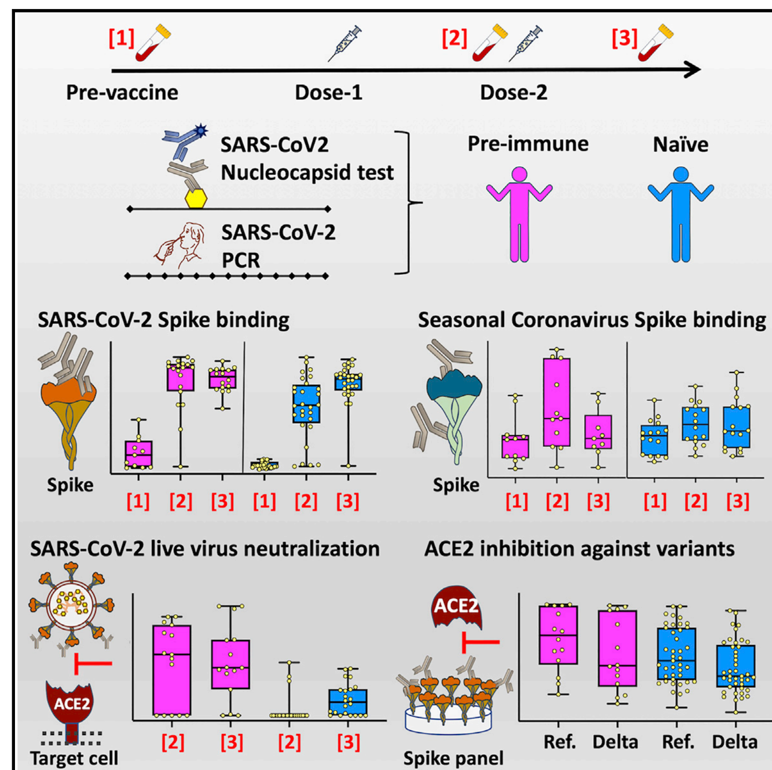
SARS-CoV-2 mRNA vaccine induces robust specific and cross-reactive IgG and unequal neutralizing antibodies in naive and previously infected people

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

Authors

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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.

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

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SUMMARY

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.

INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus causes a spectrum of disease from asymptomatic to severe forms with high mortality. The nucleocapsid protein encapsulating viral RNA and the surface exposed spike protein are the primary targets of human antibodies. The spike protein of SARS-CoV-2 mediates virus attachment and entry into host cells. It comprises a highly variable S1 segment, which harbors the N-terminal domain (NTD) and the receptor-binding domain (RBD), and a more conserved S2 segment, which includes the fusion peptide and heptad repeats required for virus fusion to host cells. Neutralizing antibody response is at present the best correlate of protection (Earle et al., 2021). However, the adaptive immune response to SARS-CoV-2 infection is variable (Chvatal-Medina et al., 2021; Gao et al., 2021; Sette and Crotty, 2021). RBD accounts for ~90% of the neutralizing activity in SARS-CoV-2 immune sera (Piccoli et al., 2020; Greaney et al., 2021a). RBD-specific antibodies target distinct antigenic sites and exert neutralizing activity principally by interfering with spike protein interactions with its cognate receptor, angiotensin-converting enzyme 2 (ACE2). A subset of the NTD-specific antibody also neutralizes SARS-CoV-2 by targeting a supersite, possibly preventing proteolytic activation, membrane fusion, or spike protein interactions with an auxiliary receptor (McCallum et al., 2021). The spike protein has been targeted in most SARS-CoV-2 vaccines under development and in those approved and currently being administered worldwide.

The recent effort to achieve widespread vaccination against SARS-CoV-2 has left in its wake a host of questions about whether the vaccine can protect against SARS-CoV-2 infection and whether the vaccine can boost immunity in previously infected people (Fakhroo et al., 2020; LoPresti et al., 2020; Miller et al., 2020). Infection with four human endemic coronaviruses (HCoVs; OC43, HKU-1, NL63, and 229E) are quite common, and most adults have antibodies to these viruses (LoPresti et al., 2020; Miller et al., 2020). Induction of cross-reactive HCoV antibodies has been reported in SARS-CoV-2 infection, and after vaccination (Röltgen et al., 2021). While their role in protection or immunopathogenesis remains unclear (Anderson et al.,

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2021; Greenbaum et al., 2021), 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

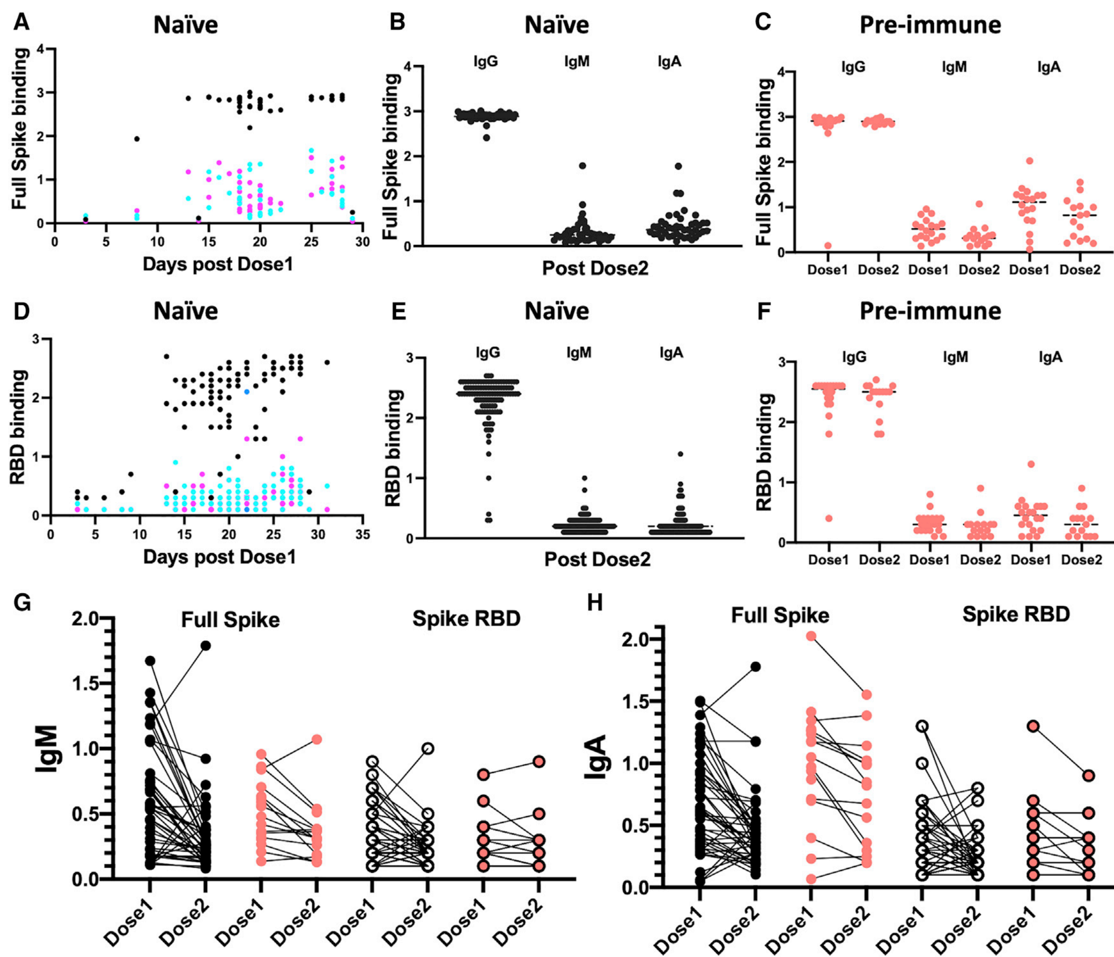

Figure 1. Antibody isotype profiling after mRNA vaccination in naive and previously infected adults

(A–F) The analysis of serum binding antibodies to (A) full spike (n = 43) and (D) spike RBD (n = 111) after dose 1 in naive individuals with the samples collected at different time points from 3 to 31 days. IgG, black; IgM, pink; IgA, cyan. Levels of IgG, IgM, and IgA antibodies binding to (B) full spike (n = 43) and (E) RBD (n = 111) in the samples collected from 20 to 66 days after dose 2 in naive individuals. Levels of IgG, IgM, and IgA antibodies binding to (C) full spike and (F) RBD in the samples collected from 6 to 28 days after dose 1 and from 35 to 65 days after dose 2 in 20 previously infected individuals.

(G and H) Changes in (G) IgM and (H) IgA antibody levels to full spike and RBD after dose 1 and 2 in naive and previously infected individuals. Changes in antibodies to full spike (n = 42, black dots) or RBD (n = 75, white-filled black circle) in naive individuals were analyzed by ELISA using the paired samples collected from 3 to 29 days after dose 1 and from 20 to 66 days after dose 2. Changes in antibodies to full spike (n = 15, red dots) or RBD (n = 15, red-filled black circle) in previously infected individuals were analyzed by ELISA using the paired samples collected from 6 to 28 days after dose 1 and from 35 to 65 days after dose 2. Each dot represents a single donor at a time point. The median is shown as a dotted line. ELISA binding is shown as OD units at 450 nm.

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.

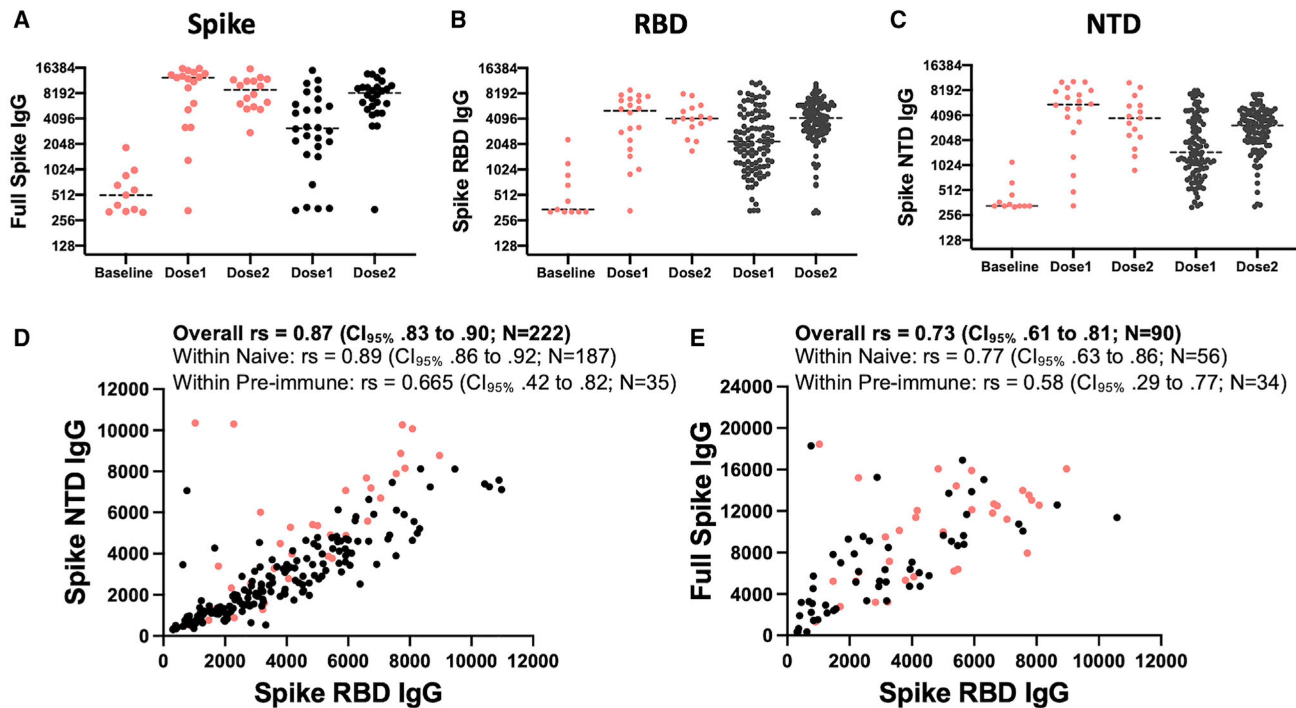

Figure 2. Analysis of spike IgG antibodies after mRNA vaccination in naive and previously infected adults

(A–C) Binding IgG levels to (A) full spike, (B) spike RBD, and (C) spike NTD were measured by ELISA using serially diluted sera from naive and previously infected individuals collected from 9 to 28 days after dose 1 and from 35 to 66 days after dose 2. Black dots, full spike (n = 28) or RBD (n = 75) or NTD (n = 75) antibodies in naive individuals; red dots, full spike (n = 20) or RBD (n = 20) or NTD (n = 20) antibodies in previously infected individuals.

(D and E) (D) Correlation of binding IgG antibodies between NTD and RBD, and (E) between RBD and full spike. The non-parametric Spearman correlation coefficient (rs) and the associated 95% confidence interval are shown for previously infected and naive individuals. Binding antibody titers are expressed as AUC. Each dot represents a single donor at a time point. The median is shown as a dotted line.

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). In general,

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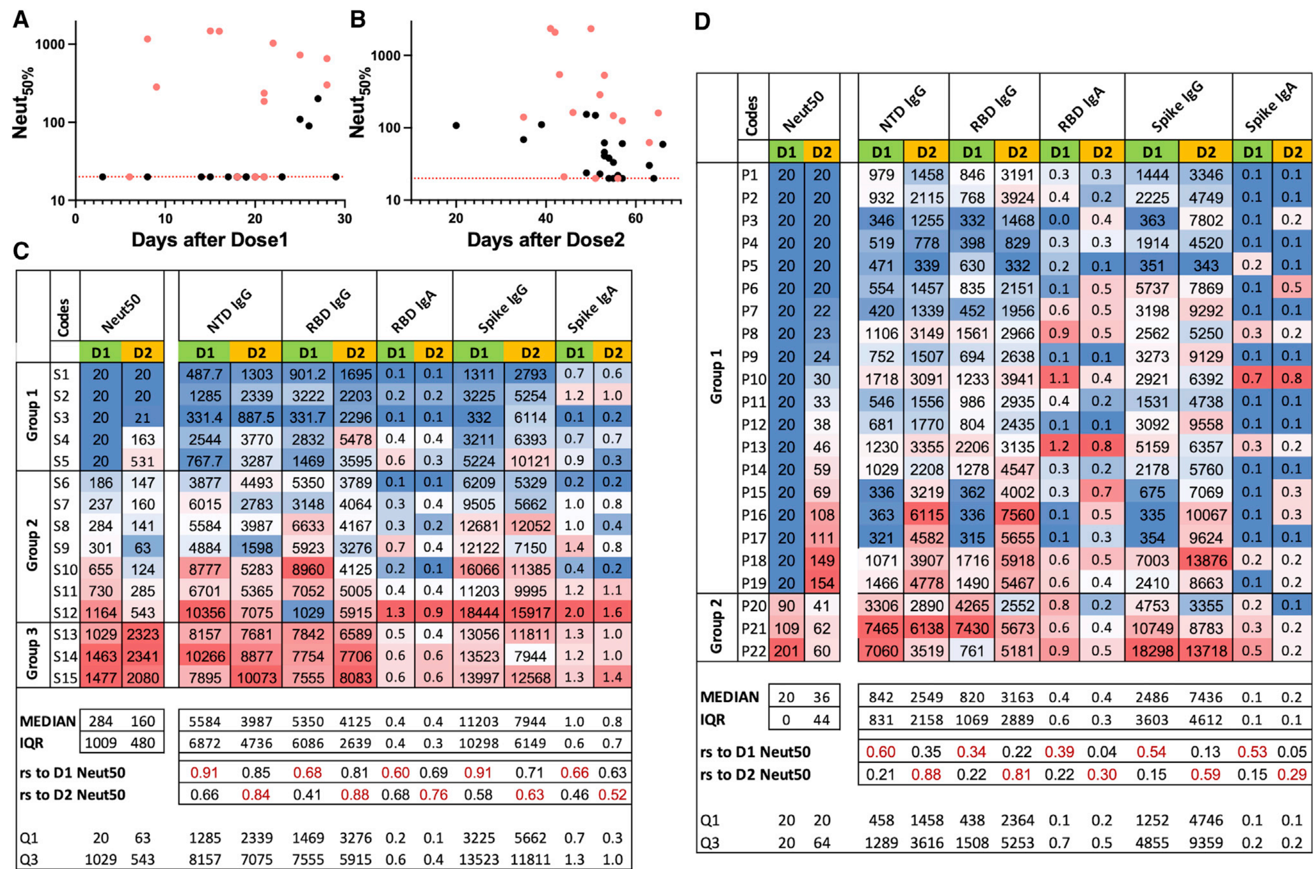

C

Group	Codes	Neut50 D1	Neut50 D2	NTD IgG D1	NTD IgG D2	RBD IgG D1	RBD IgG D2	RBD IgA D1	RBD IgA D2	Spike IgG D1	Spike IgG D2	Spike IgA D1	Spike IgA D2
Group 1	S1	20	20	487.7	1303	901.2	1695	0.1	0.1	1311	2793	0.7	0.6
	S2	20	20	1285	2339	3222	2203	0.2	0.2	3225	5254	1.2	1.0
	S3	20	21	331.4	887.5	331.7	2296	0.1	0.1	332	6114	0.1	0.2
	S4	20	163	2544	3770	2832	5478	0.4	0.4	3211	6393	0.7	0.7
	S5	20	531	767.7	3287	1469	3595	0.6	0.3	5224	10121	0.9	0.3
Group 2	S6	186	147	3877	4493	5350	3789	0.1	0.1	6209	5329	0.2	0.2
	S7	237	160	6015	2783	3148	4064	0.3	0.4	9505	5662	1.0	0.8
	S8	284	141	5584	3987	6633	4167	0.3	0.2	12681	12052	1.0	0.4
	S9	301	63	4884	1598	5923	3276	0.7	0.4	12122	7150	1.4	0.8
	S10	655	124	8777	5283	8960	4125	0.2	0.1	16066	11385	0.4	0.2
	S11	730	285	6701	5365	7052	5005	0.4	0.4	11203	9995	1.2	1.1
	S12	1164	543	10356	7075	1029	5915	1.3	0.9	18444	15917	2.0	1.6
Group 3	S13	1029	2323	8157	7681	7842	6589	0.5	0.4	13056	11811	1.3	1.0
	S14	1463	2341	10266	8877	7754	7706	0.6	0.6	13523	7944	1.2	1.0
	S15	1477	2080	7895	10073	7555	8083	0.6	0.6	13997	12568	1.3	1.4
MEDIAN		284	160	5584	3987	5350	4125	0.4	0.4	11203	7944	1.0	0.8
IQR		1009	480	6872	4736	6086	2639	0.4	0.3	10298	6149	0.6	0.7
rs to D1 Neut50				0.91	0.85	0.68	0.81	0.60	0.69	0.91	0.71	0.66	0.63
rs to D2 Neut50				0.66	0.84	0.41	0.88	0.68	0.76	0.58	0.63	0.46	0.52
Q1		20	63	1285	2339	1469	3276	0.2	0.1	3225	5662	0.7	0.3
Q3		1029	543	8157	7075	7555	5915	0.6	0.4	13523	11811	1.3	1.0

D

Group	Codes	Neut50 D1	Neut50 D2	NTD IgG D1	NTD IgG D2	RBD IgG D1	RBD IgG D2	RBD IgA D1	RBD IgA D2	Spike IgG D1	Spike IgG D2	Spike IgA D1	Spike IgA D2
Group 1	P1	20	20	979	1458	846	3191	0.3	0.3	1444	3346	0.1	0.1
	P2	20	20	932	2115	768	3924	0.4	0.2	2225	4749	0.1	0.1
	P3	20	20	346	1255	332	1468	0.0	0.4	363	7802	0.1	0.2
	P4	20	20	519	778	398	829	0.3	0.3	1914	4520	0.1	0.1
	P5	20	20	471	339	630	332	0.2	0.1	351	343	0.2	0.1
	P6	20	20	554	1457	835	2151	0.1	0.5	5737	7869	0.1	0.5
	P7	20	22	420	1339	452	1956	0.6	0.5	3198	9292	0.1	0.1
	P8	20	23	1106	3149	1561	2966	0.9	0.5	2562	5250	0.3	0.2
	P9	20	24	752	1507	694	2638	0.1	0.1	3273	9129	0.1	0.1
	P10	20	30	1718	3091	1233	3941	1.1	0.4	2921	6392	0.7	0.8
	P11	20	33	546	1556	986	2935	0.4	0.2	1531	4738	0.1	0.1
	P12	20	38	681	1770	804	2435	0.1	0.1	3092	9558	0.1	0.1
	P13	20	46	1230	3355	2206	3135	1.2	0.8	5159	6357	0.3	0.2
	P14	20	59	1029	2208	1278	4547	0.3	0.2	2178	5760	0.1	0.1
	P15	20	69	336	3219	362	4002	0.3	0.7	675	7069	0.1	0.3
	P16	20	108	363	6115	336	7560	0.1	0.5	335	10067	0.1	0.3
	P17	20	111	321	4582	315	5655	0.1	0.3	354	9624	0.1	0.1
	P18	20	149	1071	3907	1716	5918	0.6	0.5	7003	13876	0.2	0.2
	P19	20	154	1466	4778	1490	5467	0.6	0.4	2410	8663	0.1	0.2
Group 2	P20	90	41	3306	2890	4265	2552	0.8	0.2	4753	3355	0.2	0.1
	P21	109	62	7465	6138	7430	5673	0.6	0.4	10749	8783	0.3	0.2
	P22	201	60	7060	3519	761	5181	0.9	0.5	18298	13718	0.5	0.2
MEDIAN		20	36	842	2549	820	3163	0.4	0.4	2486	7436	0.1	0.2
IQR		0	44	831	2158	1069	2889	0.6	0.3	3603	4612	0.1	0.1
rs to D1 Neut50				0.60	0.35	0.34	0.22	0.39	0.04	0.54	0.13	0.53	0.05
rs to D2 Neut50				0.21	0.88	0.22	0.81	0.22	0.30	0.15	0.59	0.15	0.29
Q1		20	20	458	1458	438	2364	0.1	0.2	1252	4746	0.1	0.1
Q3		20	64	1289	3616	1508	5253	0.7	0.5	4855	9359	0.2	0.2

Figure 3. Analysis of SARS-CoV-2 live virus neutralizing antibody titers after mRNA vaccination in naive and previously infected adults

(A and B) Analysis of live virus neutralizing antibodies against reference strain (A) after dose 1 and (B) after dose 2 in previously infected and naive individuals. Previously infected (red, n = 15) participant samples were collected at different time points from 6 to 28 days after dose 1 and from 35 to 66 days after dose 2. Naive individuals' (black, n = 22) samples were collected at different time points from 3 to 29 days after dose 1 and from 20 to 66 days after dose 2. Data represent mean neutralizing antibody titers (Neut50) from duplicate measurement for a single donor at a time point.

(C and D) Heatmap showing the comparison of the neutralizing antibody titers to spike-binding antibodies in (C) 15 previously infected and (D) 22 naive individuals after dose 1 and dose 2. Median, IQR, and the non-parametric Spearman correlation coefficient (rs) are shown for each group. Group stratification by neutralizing antibodies (group 1, undetectable after dose 1 and remained undetectable or became detectable after dose 2; group 2, declined between doses 1 and 2; group 3, improved between doses 1 and 2) are shown. Analysis of vaccine-induced antibodies by age within group 1 is presented in Figure S4.

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 the 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

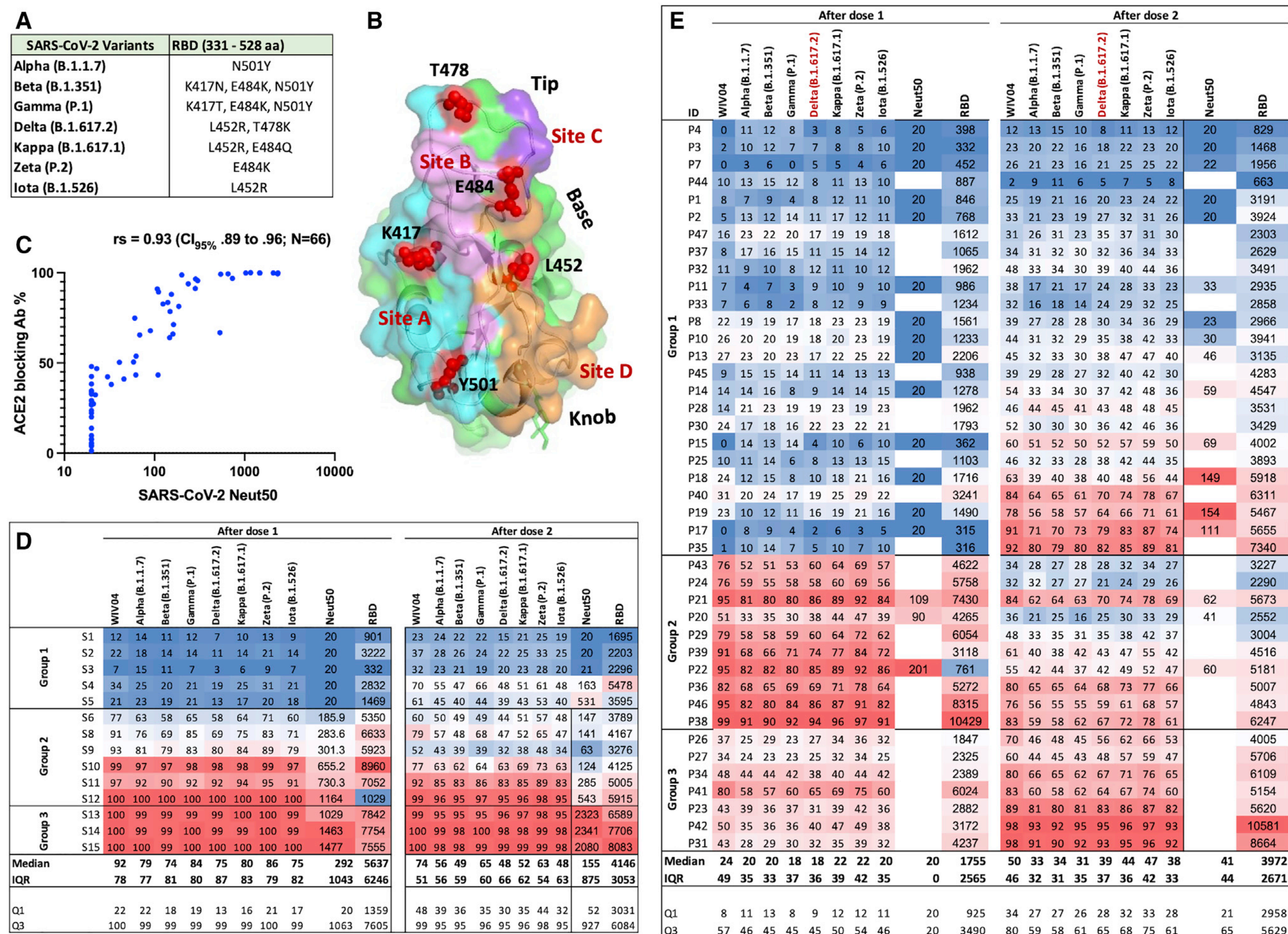

Figure 4. Analysis of mRNA-vaccine-induced ACE2 blocking antibody response against circulating variants

(A) Amino acid substitutions observed within the spike RBD among seven circulating variants.

(B) Surface representation of RBD ACE2-binding site with the overlapping epitopes targeted by major classes of neutralizing antibodies. The three ACE2-binding regions (top, base, and knob) and the overlapping epitopes (sites A, B, C, and D), classified by Wilson and co-workers (Yuan et al., 2021), are shown. Emergent mutations in the ACE2-binding site of circulating variants are shown in red ball and stick representation.

(C–E) (C) Correlation between ACE2-RBD blocking antibodies and SARS-CoV-2 live virus-neutralization titer. The non-parametric Spearman correlation coefficient (rs) and 95% confidence interval are shown for 66 samples. Heatmap showing the comparison of ACE2 blocking activities against referent and the circulating variants in (D) previously infected and (E) naive individuals after dose 1 and dose 2. Samples from 14 previously infected individuals were tested in surrogate neutralization assay using the samples collected from 15 to 28 days after dose 1 (with the exception of S3 [6 days], S12 [8 days], and S8 [9 days] samples) and from 35 to 63 days after dose 2. Samples from 42 naive individuals were also tested similarly with the samples collected from 15 to 29 days after dose 1 (with the exception of P15 [8 days], P17 [3 days], P35 [8 days], and P22 [13 days]) and from 26 to 66 days after dose 2. Data represent mean ACE2 blocking antibody (%) from duplicate measurement for a single donor at a time point. RBD-binding IgG titers (AUC) and live virus-neutralization titers (Neut50) for the reference strain are shown for comparison. Median and IQR are shown. Group stratification by neutralizing antibodies (group 1, undetectable after dose 1 and remained undetectable or became detectable after dose 2; group 2, declined between doses 1 and 2; group 3, improved between doses 1 and 2) are shown.

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 symptomatic SARS-CoV-2 infections (Premkumar et al., 2020; Röltgen et al., 2020; Wajnberg et al., 2020; Dogan et al., 2021; Freeman et al., 2021). Among convalescent plasma donors

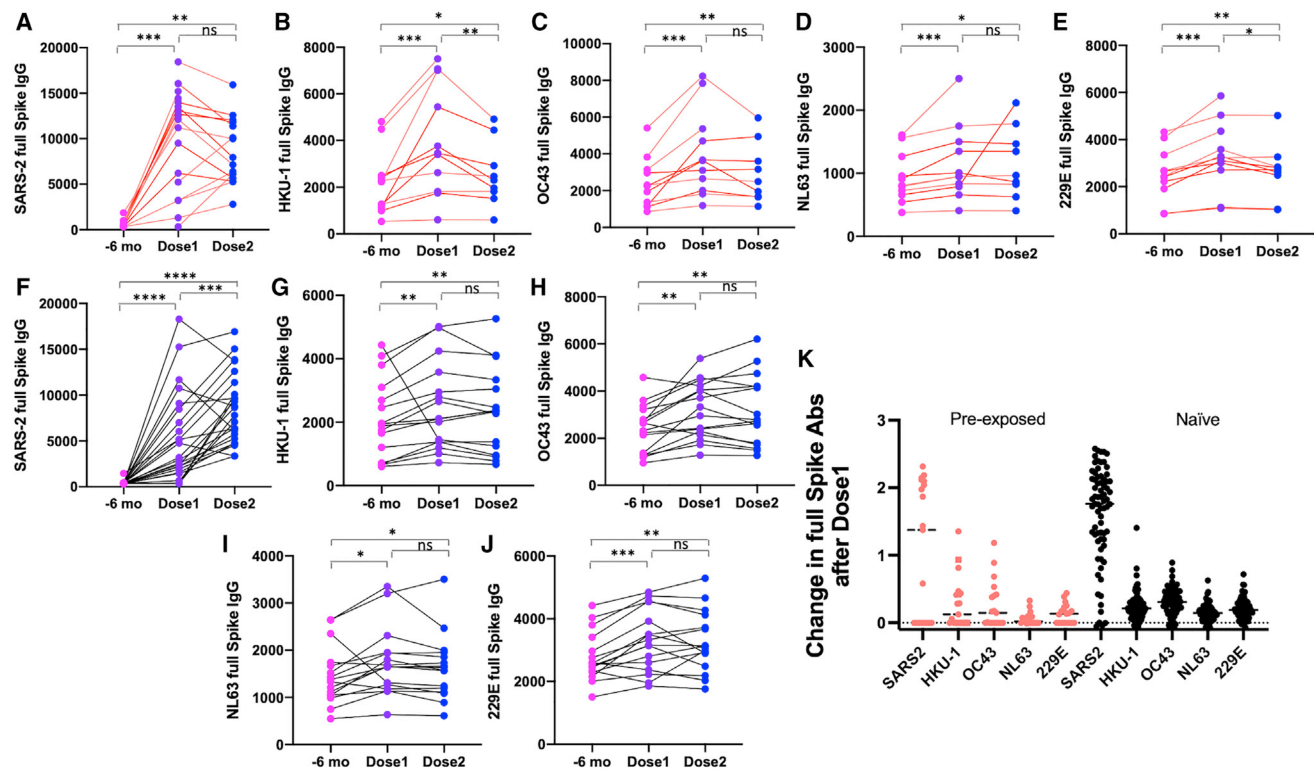

Figure 5. Longitudinal analysis of cross-reactive antibody response against HCoVs after mRNA vaccination

(A–J) Analysis of IgG-binding levels to full spike protein from (A, F) SARS-CoV-2, (B, G) HKU-1, (C, H) OC43, (D, I) NL63, and (E, J) 229E at pre-vaccine, and after doses 1 and 2. IgG antibodies to full spike proteins from seasonal coronavirus were measured by ELISA using serially diluted longitudinal sera from 16 naive (black line) and 11 previously infected (red line) individuals collected ~6 months before dose 1, after dose 1 (3–28 days), and after dose 2 (20–66 days). For comparison, SARS-CoV-2 spike IgG levels in 24 naive (black line) and 19 previously infected (red line) individuals are presented. Binding antibody titers are expressed as AUC. Wilcoxon-non-parametric paired test was performed to determine if antibody response to SARS-CoV-2 and HCoVs spike proteins between baseline and after doses are different in a subset of previously infected (A–E) and naive (F–J) individuals. The test result revealed that the antibody development between baseline and after vaccination in naive and previously infected individuals is statistically significant. The p value summary from the Wilcoxon test is shown above the scatterplot. ****$p < 0.0001$; ***0.0002; **0.0021; *0.0332; ns, 0.1234.

(K) Change in spike antibody levels (OD) between pre-vaccine and after vaccination in previously infected ($n = 19$) and naive individuals ($n = 44$). Each dot represents a single donor at a time point. The median is shown as a dotted line.

(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 antibodies 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 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; Dispinseri 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 (Röltgen 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 (Dispinseri 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 (Dispinseri 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 (PASC) 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 (Alkhansa 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'*Iq*	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 (naïve 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% CO_2 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.

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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: P0DTC2.1) 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 trimer, a codon-optimized gene was synthesized to encode for a prefusion-stabilized SARS-CoV-2 spike protein (16-1208 amino acids, Accession: P0DTC2.1) with an N-terminal human serum albumin secretion signal peptide and C-terminal T4 foldon trimerization domain, TEV protease cleavage site, His_8 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 pαH. 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 microtiter 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-BLOCKER™ (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 (Diaago 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 microtiter 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 IgG, IgM, or IgA was used (Jackson ImmunoResearch). The plate was washed three times using wash buffer, then 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 $2x10^4$ cells/well in a black 96-well clear bottom plate (Corning). Heat-inactivated serum was diluted 1:20 initially, 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% CO_2 for 1 hour. Following incubation, serum-virus complexes were added to the plated Vero E6 cells and incubated for 48 hours at 37C with 5% CO_2. 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.