Supplementary Appendix

Supplement to: Schmidt F, Muecksch F, Weisblum Y, et al. Plasma neutralization of the SARS-CoV-2 omicron variant. N Engl J Med. DOI: 10.1056/NEJMc2119641

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

Supplementary Rationale

The recent emergence of the B.1.1.159 (Omicron) variant of SARS-CoV-2 [1-3] has engendered widespread concern. Omicron has spread internationally and is responsible for rapidly increasing case numbers, particularly in South Africa[3]. Although the pathogenic potential of the new variant remains unclear[3], a striking feature is the large number of amino acid substitutions, insertions and deletions in the spike protein (32 of the total of ~50 nonsynonymous changes in the viral genome)[1] suggesting adaptation to substantial selective pressure. Some of the substitutions, for example those proximal to the furin cleavage site, are thought to be fitness enhancing and may facilitate virus spread[4], but the majority of the changes are expected to reduce neutralizing antibody recognition.

The number of neutralizing epitopes targeted by polyclonal antibodies in SARS-CoV-2 convalescent or vaccinated individuals is an important determinant of the genetic barrier to viral escape[5]. Whereas single monoclonal antibodies are vulnerable to spike escape mutations, combinations targeting non-overlapping epitopes are more resistant to such changes[6,7]. There are numerous antibody targets in the SARS-CoV-2 spike protein, but polyclonal neutralizing responses are dominated by antibodies to the receptor binding domain (RBD) and the N-terminal domain (NTD) of spike[5,8-10]. Indeed, aggregation of ~20 RBD and NTD mutations in a polymutant spike protein (PMS20) was required for evasion of polyclonal antibodies elicited in the majority of individuals who had been infected, or who had received two doses of an mRNA vaccine[5,15]. Notably, several of the changes in the PMS20 spike are the same or similar to the changes in the emergent Omicron variant spike[11,12] (Figure S1A, B), leading to the prediction that Omicron would exhibit substantial antigenic escape.

Affinity maturation of individual SARS-CoV-2 neutralizing antibodies can dramatically alter their properties in ways that are pertinent for the emergence and control of variants[13-16]. The number

of antibody variable region mutations and the binding affinity of antibodies increases over months and can vary depending on the nature of SARS-CoV-2 antigen exposure[13,15-18]. Indeed, affinity maturation can markedly expand SARS-CoV-2 neutralizing antibody breadth, enabling neutralization of SARS-CoV-2 variants that escape neutralization by corresponding ancestral antibodies and imposing a requirement for multiple amino acid substitutions for escape[7,13-15,18,19]. Thus, Omicron has emerged in the context of a globally heterogeneous and evolving neutralizing antibody response in host populations that might provide varying degrees of protection, depending on infection and vaccination history. Here, we determined the ability of individuals with varying exposure to SARS-CoV-2 infection and vaccination, to neutralize SARS-CoV-2 pseudotypes with spike proteins corresponding to the parental virus used in vaccine immunogens, PMS20 or the emergent Omicron variant.

Supplementary Description of Results

We measured titers of convalescent individuals who had been infected early in the pandemic, and whose plasma was donated an average of 1.3 and 6.2 months after infection (Supplementary Table S2). These individuals subsequently received one or two doses of an mRNA vaccine (Pfizer/BNT or Moderna) before collection of additional plasma samples approximately 12 months after initial infection. For comparison, we measured neutralizing titers in a group of convalescent individuals from the same initial cohort who were not vaccinated (Supplementary Table S2). We also measured neutralizing titers in uninfected individuals that received 2 doses of an mRNA vaccine (Pfizer/BNT or Moderna) and donated plasma at 1.2 and 5 months after the second dose. These same individuals received a third Pfizer/BNT dose booster >6 months after the second dose and donated another plasma sample 1 month after the third dose (Supplementary Table S3). Finally, we examined a cohort that received a single dose of an Ad26 adenovirus vaccine (J&J) that has been widely deployed in the USA, at ~1 and 5 months after vaccination (Supplementary Table S3).

NEUTRALIZATION OF PMS20 AND OMICRON PSEUDOTYPES BY CONVALESCENT PLASMA

Like PMS20, the Omicron variant pseudotyped virus was substantially resistant to neutralization by plasma compared to Wuhan-hu-1. In convalescent individuals whose plasmas were collected at ~1.2 months after infection, the median and range of the NT_{50} values was 2616 (681-19450), 49 (<25-429) and 68 (29-667) for the Wuhan-hu-1, PMS20 and Omicron spike proteins respectively (Figure S2A). After 6 months of convalescence, the median and range of NT_{50} values from the same individuals was 1678 (321 - 5189), 28 (<25 - 248), and 42 (<25 - 428) for the Wuhan-hu-1, PMS20 and Omicron spike proteins respectively. (Figure S2B). Similarly, plasmas collected from different individuals in the same cohort, 1 year after infection had NT_{50} values (median and range) of 2037 (127 - 25835), 76 (<25 - 907) and 136 (<25 - 667) for Wuhan-hu-1, PMS20 and Omicron pseudotypes (Figure S2C). Overall, individuals who were infected but not vaccinated show substantially reduced plasma neutralizing activity including many with sub detectable titers against Omicron pseudotypes.

NEUTRALIZATION OF PMS20 AND OMICRON PSEUDOTYPES BY VACCINE RECIPIENT PLASMA

Plasmas from vaccinated individuals also exhibited substantially impaired ability to neutralize the Omicron variant. In plasmas from individuals who received mRNA vaccines ~1.3m prior to sampling, the median and range of the NT_{50} values were 7627 (2299 - 50640), for Wuhan-hu- 1, but only 60 (<25 - 201) for PMS20 and 92 (25 - 327) for Omicron (Figure S3A). These values correspond to mean (±SD) loss of potency of 187±24 fold for PMS20 and 127±66 fold for Omicron. At 5 months after vaccination, neutralizing titer (median and range) in plasmas from the same individuals had waned to 2435 (1117 – 6228) for Wuhan-hu-1 and were as low as 43

(<25 – 108) for PMS20 and 126 (27 – 321) for Omicron, corresponding to mean (±SD) reductions in NT_{50} of 58±23-fold for PMS20 and 27±17-fold for Omicron (Figure S3B).

Plasmas from single dose J&J Ad26 vaccine recipients were particularly poor at neutralizing the variants, with many having undetectable neutralizing activity against these pseudotypes. Indeed, Plasma collected 1 month after single dose Ad26 J&J vaccination had median (and range) NT_{50} values of 588 (167 - 4177), <25 (<25-314) and <25 (<25-201) for the Wuhan-hu-1, PMS20 and Omicron spike proteins respectively (Figure S3C). NT_{50} values for J&J Ad26 recipients were quite stable or even increased in some cases over time, such that at five months after the single dose J&J Ad26 vaccination, NT_{50} values were 982 (52 – 5597), 36 (<25 – 378) and 43 (<25 – 368) for the Wuhan-hu-1, PMS20 and Omicron pseudotypes respectively (Figure S3D).

EFFECTS OF mRNA VACCINE BOOSTERS ON NEUTRALIZATION BY PLASMA FROM CONVALESCENT OR PREVIOUSLY mRNA VACCINATED INDIVIDUALS

Vaccination of previously infected individuals has previously been shown to substantially elevate neutralizing titers and breadth[5,20,21]. Vaccination of convalescent individuals[5,15] or boosting of an initial vaccine response by administration of a third mRNA (Pfizer/BNT) vaccine dose >6 months after the initial series led to the acquisition of substantial neutralizing activity against PMS20 and against Omicron (Figure S4A, B). Specifically, in individuals that were previously infected by SARS-CoV-2 and later vaccinated, the median and range of the NT_{50} values was 388872 (98522 - 1304453), 14982 (1699 - 448699) and 8106 (1503 - 56537) for the Wuhan-hu-1, PMS20 and Omicron spike protein pseudotypes respectively (Figure S4A).

For individuals that were vaccinated with 2 doses of a Pfizer/BNT or Moderna mRNA vaccines ~6 months previously and then boosted with a third mRNA (Pfizer/BNT) dose ~1month prior to

sampling, the median and range of the NT_{50} values was 65617 (15641 - 341247), 1505 (404 - 9039) and 3871 (1411 - 21300) for the Wuhan-hu-1, PMS20 and Omicron pseudotypes (Figure S4B)

Supplemental Discussion

Compared with previous naturally occurring SARS-CoV-2 variants, Omicron exhibits an unprecedented degree of neutralizing antibody escape. Indeed, the degree of neutralization resistance exhibited by Omicron is similar to that exhibited by PMS20, a designed neutralization resistant spike in which 20 naturally occurring and laboratory selected mutations were aggregated[5]. The similarity in neutralization properties and distribution of changes on the spike protein surface between PMS20 and Omicron argues that a major selective pressure leading to the emergence of Omicron was imposed by neutralizing antibodies. Whether this selective pressure occurred in one or more immunocompromised individuals with persistent infection, or populations that have experienced high prevalence infection by waves of prior variants remains unclear.

Of particular concern, neutralizing antibody titers against Omicron were low, even below the limit of detection in a significant fraction of convalescent individuals, Ad26 vaccine recipients or 2 dose mRNA vaccine recipients, particularly following the waning that ensues following infection or vaccination. Nevertheless, individuals that had been previously infected with SARS-CoV-2 and subsequently received mRNA vaccines, or those that have received three doses of mRNA vaccines had substantial neutralizing antibody titers against Omicron ~1month after boosting. The ability of plasmas from these individuals to neutralize Omicron likely represents the combined effect of increased antibody levels following multiple exposures to antigen, as well as the effects of affinity maturation that can dramatically improve the neutralizing breadth of individual SARS-CoV-2 antibodies as well as polyclonal plasma[5,15].

Limitations of this study include the number of participants (18-20 per group) and uncertainty in the relationship between neutralizing antibody titers and vaccine efficacy, particularly as circulating SARS-CoV-2 variants diverge from the Wuhan-hu-1 sequence to which the convalescent and vaccinated individuals in this study were exposed. The contribution of cell mediated immunity to protection may further degrade the predictive values of neutralizing antibody measurements. Additionally, the acquisition of breadth following the boosting of convalescent or mRNA vaccine immunity was assessed shortly after boosting. The longevity of these the neutralizing potency and breadth in in the context of an evolving pandemic is yet to be determined.

Nevertheless, these findings suggest that boosting and promoting affinity maturation of the antibodies of those who have previously been infected or vaccinated using existing Wuhan-hu-1 based vaccine immunogens will provide additional protection against Omicron variant infection and disease. The emergence of the Omicron lineage that is phylogenetically distinct from the previously globally dominant Delta SARS-CoV-2 variant also illustrates the need for continuing vigilance and mitigation of virus transmission. The successive emergence, and global spread of the Alpha, Delta, and Omicron variants underscores our inability to predict the future emergence of variants that might facilitate targeted vaccine development. The findings described herein and the emergence of Omicron suggest that effort should be devoted to development of vaccination strategies that are broadly based rather than, or in addition to, those narrowly targeted at contemporary emergent SARS-CoV-2 variants.

Methods

PLASMA SAMPLES

The 169 plasma samples were from the following three longitudinal cohorts (Table S1): (i) Convalescent individuals were randomly selected from a previously described cohort of volunteers with PCR confirmed SARS-CoV-2 infection[9]. The severity of acute infection was assessed by the World Health Organization (WHO) 'Ordinal Clinical Progression/Improvement Scale' (https://www.who.int/publications/i/item/ covid-19-therapeutic-trial-synopsis). After recruitment, some volunteers receive 2 doses of the Pfizer/BNT or Moderna mRNA vaccine between 6 months and 12 months after infection[15]; (ii) Uninfected vaccine recipients were randomly selected from previously described cohorts of volunteers with no history of prior SARS-CoV-2 infection who received 3 doses of the Pfizer/BNT mRNA vaccine[15,17]; (iii) Volunteers with no history of prior SARS-CoV-2 infection who received the single-dose J&J Ad26 vaccine. Plasma samples were collected approximately 1, 5-6 and/or 12 months after initial vaccination or infection as detailed in Tables S2 and S3. Individuals were selected for this study at random with blinding with respect to antibody binding, neutralization titer, or donor demographic os symptom characteristics. The study visits and blood draws were reviewed and approved by the Institutional Review Board of the Rockefeller University (IRB no. DRO-1006, 'Peripheral Blood of Coronavirus Survivors to Identify Virus-Neutralizing Antibodies').

PSEUDOTYPE NEUTRALIZATION ASSAY

The Omicron spike coding sequence was derived from sequence ID EPI_ISL_6640919. It was codon-optimized and synthesized as a C-terminally truncated Δ19 form in nine fragments (IDT). We also introduced a furin cleavage site mutation (R683G) that does not change the neutralization properties of the SASR-CoV-2 spike protein but enables higher titer pseudotyped viral stocks to be generated from transfected cells5. These synthetic DNA fragments, ranging in size from 444-599bp and a NheI/XbaI-linearized pCR3.1 plasmid were Gibson assembled via

40bps overlapping sequences. Individual plasmid clones were completely sequenced (Illumina MiSeq) and a single correct clone was used in these studies. The Wuhan-hu-1 and PMS20 spike proteins were previously described5,21.

Neutralizing titers were measured using a SARS-CoV-2 pseudotyped HIV-1-based assay that recapitulates neutralizing titers obtained with authentic SARS-CoV-221. Plasmas were serially diluted (five-fold dilution interval) and then incubated with a SARS-CoV-2 spike (Wuhan-hu-1, PMS20 or Omicron) pseudotyped HIV-1 based nanoluc luciferase reporter virus for 1 hour at 37 °C. The pseudotyped virus and antibody mixture was transferred to 96 well plates containing HT1080/ACE2.cl14 cells. After 48 hours, the cells were washed with PBS and lysed with Luciferase Cell Culture Lysis reagent (Promega). Then Nanoluc Luciferase activity in cell lysates was measured using the Nano-Glo Luciferase Assay System and a Glomax Navigator luminometer (Promega). The relative luminescence units were normalized to those measured in cells infected with the corresponding pseudotyped virus in the absence of plasma. The half-maximal plasma neutralizing titer (NT50) was determined using four-parameter nonlinear regression (least squares regression method without weighting) (GraphPad Prism). The NT50 for each plasma was measured twice in two independent experiments, carried out by two different groups of researchers, with two technical replicates each.

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Author contributions

P.D.B., T.H., M.C.N., F.S., and F.M. conceived, designed and analyzed the experiments. F.S., Y.W., F.M., J.D.S, and E.B. performed pseudotype neutralization experiments. F.S. constructed expression plasmids. A.C. performed NGS. Y.Z., M.C, C.G. and executed clinical protocols and recruited participants and processed samples. P.D.B., T.H., and M.C.N. wrote the manuscript.

Table S1. Plasma samples tested for neutralizing activity

Plasma panel	N		Description	Time after infection or vaccination
Panel 1[1] (Figure 1, S2A)	20	Convalescent		~1m post infection
Panel 2[1] (Figure 1, S2B)	20	Convalescent		~6m post infection
Panel 3 (Figure 1, S2C)	20	Convalescent		~1y post infection
Panel 4[2] (Figure 1, S3A)	18	Vaccinated	2 doses of Pfizer mRNA	~1m post 2nd dose
Panel 5[2] (Figure 1, S3B)	18	Vaccinated	2 doses of Pfizer mRNA	~5m post 2nd dose
Panel 6[2] (Figure 1, S4A)	18	Vaccinated	3 doses of Pfizer mRNA	~1m post 3rd dose
Panel 7[3] (Figure S3C)	19	Vaccinated	1 dose J&J Ad26	~1m post single dose
Panel 8[3] (Figure S3D)	19	Vaccinated	1 dose J&J Ad26	~5m post single dose
Panel 9[1] (Figure 1, S4B)	17	Convalescent + Vaccinated	1 or 2 doses of Pfizer or Moderna mRNA, following prior SARS-CoV-2 infection	~12m post infection, ~1m post vaccination

[1]Plasma panels 1, 2, and 9 from matched convalescent individuals (See Table S2)

[2]Plasma panels 4, 5, and 6 from matched mRNA vaccinated individuals (See Table S3)

[3]Plasma panels 7 and 8 from matched J&J Ad26 vaccinated individuals (See Table S3)

Table S2. Individual convalescent participant characteristics

ID	Age (years)	Sex	Race	Ethnicity	Acute disease severity by WHO (0-8)*	Temporal dynamics (days)			Vaccination status		
						Sx onset to initial ~1m sample	Sx onset to ~6m follow up sample	Sx onset to ~1y follow up sample	Vaccine received	# of doses received prior to 1y	Days between first dose and 1y study visit
7	40	M	White	Non-Hispanic	2	30	181	376	Pfizer-BNT	2	65
20	26	F	White	Non-Hispanic	2	17	191	345	Moderna	2	35
24	34	M	White	Non-Hispanic	1	30	175	336	Pfizer-BNT	N/A	N/A
55	36	M	White	Non-Hispanic	2	49	210	349	Moderna	2	41
71	45	F	White	Non-Hispanic	2	48	202	386	Pfizer-BNT	2	62
96	48	F	White	Non-Hispanic	1	30	194	359	Pfizer-BNT	2	54
115	65	F	White	Non-Hispanic	2	41	188	335	Moderna	1	12
120	56	F	White	Non-Hispanic	1	48	207	375	Moderna	2	67
135	62	F	White	Non-Hispanic	2	31	190	341	Moderna	1	27
186	38	F	N/A	N/A	1	33	189	356	Pfizer-BNT	2	73
222	28	M	Asian	Non-Hispanic	2	37	173	347	Pfizer-BNT	2	41
230	50	M	White	Non-Hispanic	2	33	190	372	Pfizer-BNT	N/A	N/A
233	55	M	White	Non-Hispanic	2	41	206	377	Moderna	1	8
325	52	M	White	Non-Hispanic	2	38	192	353	Moderna	2	49
393	69	M	White	Non-Hispanic	5	54	187	362	Moderna	2	57
394	48	F	Multiple	Hispanic	2	67	200	375	Moderna	N/A	N/A
401	61	M	White	Non-Hispanic	2	53	209	371	Pfizer-BNT	1	18
500	46	M	White	Non-Hispanic	2	53	207	375	Pfizer-BNT	1	20
537	52	M	White	Non-Hispanic	2	45	178	357	Pfizer-BNT	1	14
539	73	F	White	Non-Hispanic	5	55	209	362	Pfizer-BNT	2	50
	Mean = 49.2 SD = 13	11M, 9F	17 White 1 Asian 1 Multiple		Mean = 2.1 SD = 1.1	Mean = 41 SD = 12 **Panel 1**	Mean = 194 SD = 12 **Panel 2**	Mean = 360 SD = 15 **Panel 9**	11 Pfizer-BNT 9 Moderna		Mean = 41 SD = 21
8	37	M	White	Non-Hispanic	2			370	N	N/A	N/A
9	35	F	White	Non-Hispanic	2			366	N	N/A	N/A
21	54	M	White	Hispanic	2			340	N	N/A	N/A
38	57	F	White	Non-Hispanic	2			337	N	N/A	N/A
40	44	M	White	Non-Hispanic	2			345	N	N/A	N/A
46	39	M	White	Non-Hispanic	2			337	N	N/A	N/A
47	43	F	White	Non-Hispanic	2			340	N	N/A	N/A
72	42	M	White	Non-Hispanic	2			352	N	N/A	N/A
75	46	F	White	Non-Hispanic	1			340	N	N/A	N/A
76	49	F	White	Non-Hispanic	1			379	N	N/A	N/A
328	54	F	White	Non-Hispanic	2			365	N	N/A	N/A
353	60	M	White	Non-Hispanic	2			366	N	N/A	N/A
403	52	M	Asian	Non-Hispanic	4			356	N	N/A	N/A
410	34	M	White	Non-Hispanic	2			349	N	N/A	N/A
437	43	F	Asian	Non-Hispanic	2			353	N	N/A	N/A
461	49	M	White	Non-Hispanic	2			350	N	N/A	N/A
501	32	M	Asian	Non-Hispanic	4			367	N	N/A	N/A
507	39	M	White	Non-Hispanic	2			361	N	N/A	N/A
547	59	M	White	Non-Hispanic	3			359	N	N/A	N/A
633	39	M	White	Non-Hispanic	1			358	N	N/A	N/A
	Mean = 45.4 SD = 8.5	13M, 7F	17 White 3 Asian		Mean = 2.1 SD = 0.8			Mean= 354 SD=12 **Panel 3**			

*= WHO Ordinal Scale for Clinical Improvement, COVID-19 Trial Design Synopsis

Table S3. Individual vaccinated participant characteristics

						Vaccine platform			Vaccination time line (days)				
ID	Age (years)	Sex	Race	Ethnicity	COVID-19 history	1st dose	2nd dose	3rd dose	1st to 2nd dose	2nd to 3rd dose	2nd (mRNA) or 1st (J&J) dose to 1st sample	2nd (mRNA) or 1st (J&J) dose to 2nd sample	3rd (mRNA) dose to Follow-up sample
C001	38	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	22	251	35	133	56
C002	41	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	373	79	177	27
C004	37	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	389	70	170	27
C005	50	M	Black	Non-Hispanic	No	Moderna	Moderna	Pfizer-BNT	28	326	91	175	94
C006	65	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	246	22	146	22
C007	27	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	252	37	141	21
C008	54	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	244	35	141	28
C009	32	F	White	Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	244	36	141	22
C010	66	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	245	34	146	21
C011	73	F	White	Non-Hispanic	No	Moderna	Moderna	Pfizer-BNT	28	274	28	131	21
C017	25	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	252	21	244	21
C018	24	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	242	48	172	23
C019	34	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	245	34	146	21
C021	37	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	212	28	168	47
C028	25	M	White	Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	252	41	168	28
C029	58	F	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	244	49	166	22
C031	26	M	White	Non-Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	21	242	37	165	28
C034	39	F	White	Hispanic	No	Pfizer-BNT	Pfizer-BNT	Pfizer-BNT	24	273	31	245	19
	Mean = 42 SD = 14	9M, 9F	17 White 1 Black	15 Non Hispanic 3 Hispanic		16 Pfizer-BNT 2 Moderna	16 Pfizer-BNT 2 Moderna		Mean = 22 SD = 2	Mean = 267 SD = 47	Mean = 42 SD = 19 **Panel 4**	Mean = 165 SD = 33 **Panel 5**	Mean = 30 SD = 18 **Panel 6**
C055	39	F	White	Non-hispanic	No	J&J	-	-	-	-	34	175	-
C056	56	F	White	Non-Hispanic	No	J&J	-	-	-	-	52	187	-
C057	27	M	White	Non-hispanic	No	J&J	-	-	-	-	44	178	-
C059	23	F	White	Non-hispanic	No	J&J	-	-	-	-	44	181	-
C060	55	M	White	Non-hispanic	No	J&J	-	-	-	-	35	191	-
C061	46	F	White	Non-Hispanic	No	J&J	-	-	-	-	27	177	-
C062	32	F	White	Non-hispanic	No	J&J	-	-	-	-	53	200	-
C064	37	F	White	Non-hispanic	No	J&J	-	-	-	-	46	178	-
C066	49	F	White	Hispanic	No	J&J	-	-	-	-	50	183	-
C067	46	F	Black	Non-hispanic	No	J&J	-	-	-	-	52	193	-
C069	51	F	White	Non- Hispanic	No	J&J	-	-	-	-	37	172	-
C070	45	M	White	Non-Hispanic	No	J&J	-	-	-	-	67	187	-
C072	45	F	White	Non-Hispanic	No	J&J	-	-	-	-	63	150	-
C073	41	F	White	Non-Hispanic	No	J&J	-	-	-	-	56	197	-
C075	32	M	White	Non-Hispanic	No	J&J	-	-	-	-	72	180	-
C076	34	M	White	Hispanic	No	J&J	-	-	-	-	72	179	-
C079	51	M	Black	Non-Hispanic	No	J&J	-	-	-	-	46	152	-
C080	35	M	White	Non-Hispanic	No	J&J	-	-	-	-	34	140	-
C081	41	M	Asian	Non- Hispanic	No	J&J	-	-	-	-	31	136	-
	Mean = 41 SD = 9	8M, 11F	16 White 2 Black 1 Asian	17 Non-Hispanic 2 Hispanic							Mean = 48 SD = 14 **Panel 7**	Mean = 176 SD = 18 **Panel 8**	

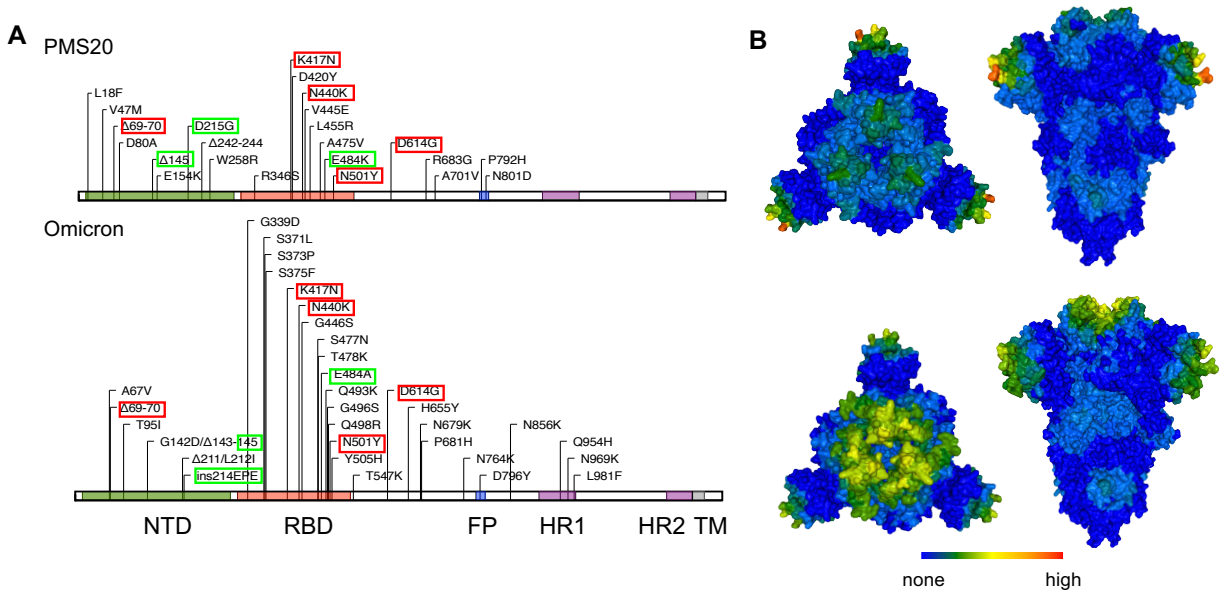

Figure S1. Distribution of mutations in the naturally emergent Omicron variant, and the neutralization resistant designed PMS20 spike proteins.
(A) Schematic diagram of PMS20 and Omicron spike proteins with amino acid changes compared to Wuhan-1 indicated. Red outlines highlight identical changes in PMS20 and Omicron, green outlines indicate nonidentical changes affecting same residues in PMS20 and Omicron. Position of spike features is marked: NTD = N-terminal domain RBD = Receptor Binding Domain, FP = Fusion Peptide, HR = heptad repeat, TM = Transmembrane.
(B) Representation of the spatial distribution of mutations on the surface of PMS20 and Omicron spike proteins. Frequency of amino acid changes relative to Wuhan-hu-1 in PMS20 or Omicron projected onto the SARS-CoV-2 spike structure (PDB 6VXX). The average frequency of substitutions in a 15 angstrom radius is represented using a color spectrum (scale 0-40) (https://biostructmap.burnet.edu.au).

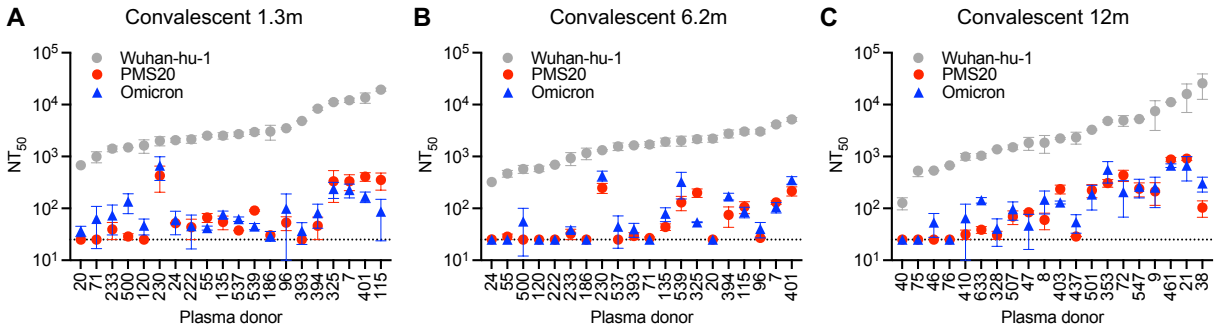

Figure S2. Plasma neutralizing titers against Wuhan-hu-1, PMS20 and Omicron SARS-CoV-2 variants in convalescent, unvaccinated individuals

(A-C) NT_{50} values of plasmas collected at 1 month (A), 6 months (B) and 12 months (C) after SARS-CoV-2 infection against Wuhan-hu-1, PMS20 and Omicron spike pseudotyped reporter viruses. Each NT_{50} was determined in two independent experiments (each with two technical replicates). The median and range of the two independent determinations is plotted. Dashed line indicates the lowest plasma dilution tested (1:25). Plasmas from the same individuals collected at 1.3m and 6.2m were tested in panels (A) and (B). Plasmas from different individuals were tested in panel (C).

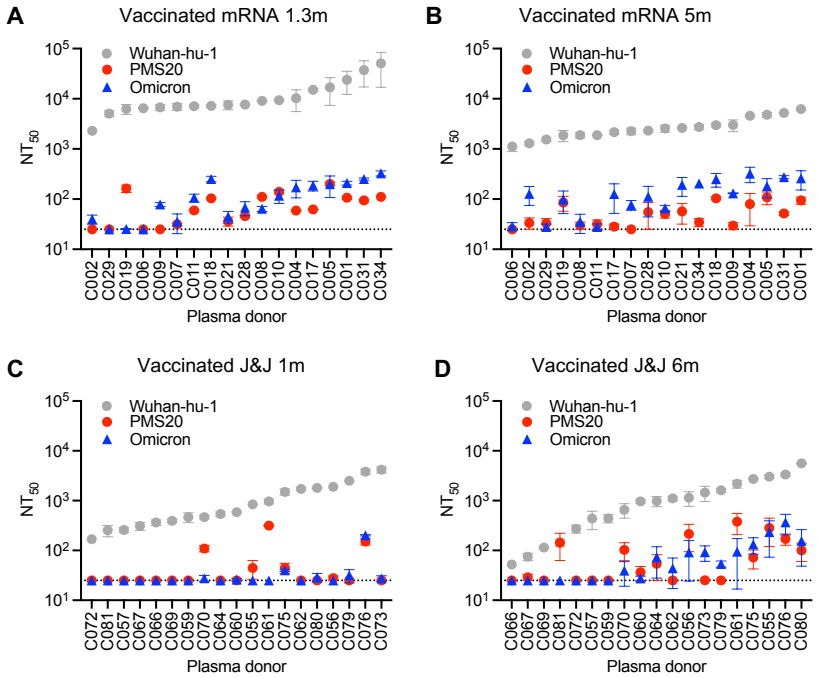

Figure S3. Plasma neutralizing titers against Wuhan-hu-1, PMS20 and Omicron SARS-CoV-2 variants in vaccine recipients

(A, B) NT_{50} values of plasmas from recipients of standard 2 dose mRNA (Pfizer/BNT or Moderna) approximately one (A) and five (B) months after vaccination. Plasmas from the same individuals were tested in (A) and (B).

(C,D) NT_{50} values of plasmas from recipients of single dose Ad26 (J&J) SARS-CoV-2 vaccines approximately one (C) and five (D) months after vaccination. Plasmas from the same individuals were tested in (C) and (D).

For each panel, NT_{50} were determined in two independent experiments (each with two technical replicates). The median and range of the two independent determinations is plotted. Dashed line indicates the lowest plasma dilution tested (1:25)

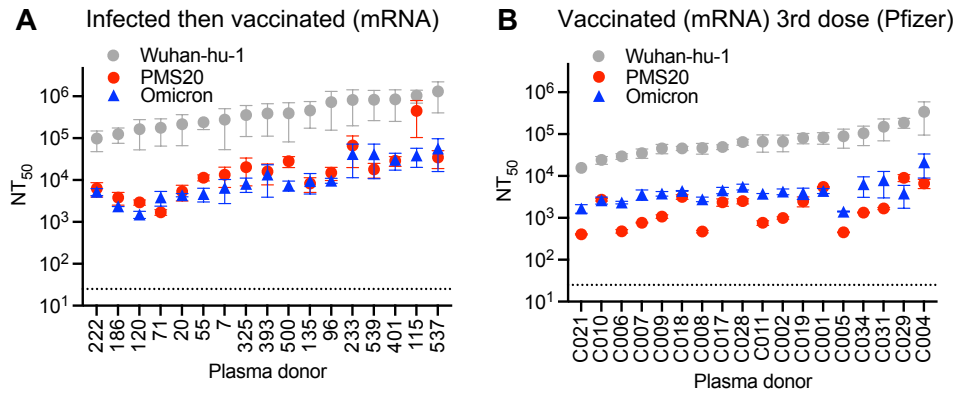

Figure S4. Effect of vaccinating previously infected individuals or boosting previously vaccinated individuals on Wuhan-hu-1, PMS20 and Omicron plasma neutralizing titers.
(A) NT_{50} values of plasmas from recipients of mRNA (Pfizer/BNT or Moderna) vaccines subsequent to infection with SARS-CoV-2. Plasmas are from the same individuals as those tested prior to vaccination in Figure S2 (A and B), and were collected ~1 year after infection and at (mean ± SD) 41 ± 21 days after vaccination (see Table S2)
(B) NT_{50} values of plasmas from recipients of three doses of mRNA (Pfizer/BNT or Moderna) vaccines with the third dose (Pfizer/BNT) administered >6m after the second and at (mean ± SD) 30 ± 18 days after the third vaccine dose (see Table S3). Plasmas are from the same individuals as those tested following two-dose vaccination, but prior to boosting, in Figure S3 (A and B).
For (A) and (B) each NT_{50} was determined in two independent experiments (each with two technical replicates). The median and range of the two independent determinations is plotted. Dashed line indicates the lowest plasma dilution tested (1:25).

References

1. https://www.gisaid.org/
2. Torjesen I. Covid-19: Omicron may be more transmissible than other variants and partly resistant to existing vaccines, scientists fear. Bmj 2021;375:n2943. (In eng). DOI: 10.1136/bmj.n2943.
3. Karim SSA, Karim QA. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. Lancet 2021 (In eng). DOI: 10.1016/s0140-6736(21)02758-6.
4. Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell 2020;78(4):779-784.e5. (In eng). DOI: 10.1016/j.molcel.2020.04.022.
5. Schmidt F, Weisblum Y, Rutkowska M, et al. High genetic barrier to SARS-CoV-2 polyclonal neutralizing antibody escape. Nature 2021 (In eng). DOI: 10.1038/s41586-021-04005-0.
6. Baum A, Fulton BO, Wloga E, et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science (New York, NY) 2020;369(6506):1014-1018. (In eng). DOI: 10.1126/science.abd0831.
7. Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. eLife 2020;9:e61312. (In eng). DOI: 10.7554/eLife.61312.
8. Liu L, Wang P, Nair MS, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 2020;584(7821):450-456. (In eng). DOI: 10.1038/s41586-020-2571-7.
9. Robbiani DF, Gaebler C, Muecksch F, et al. Convergent Antibody Responses to SARS-CoV-2 Infection in Convalescent Individuals. Nature 2020;584:437-442.
10. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell host & microbe 2021;29(3):463-476. (In eng). DOI: 10.1016/j.chom.2021.02.003.
11. Guy AJ, Irani V, Richards JS, Ramsland PA. BioStructMap: a Python tool for integration of protein structure and sequence-based features. Bioinformatics (Oxford, England) 2018;34(22):3942-3944. (In eng). DOI: 10.1093/bioinformatics/bty474.
12. Guy AJ, Irani V, Beeson JG, et al. Proteome-wide mapping of immune features onto Plasmodium protein three-dimensional structures. Scientific reports 2018;8(1):4355. (In eng). DOI: 10.1038/s41598-018-22592-3.
13. Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. Nature 2021;591:639-644. (In eng). DOI: 10.1038/s41586-021-03207-w.
14. Muecksch F, Weisblum Y, Barnes CO, et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. Immunity 2021 (In eng). DOI: 10.1016/j.immuni.2021.07.008.
15. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature 2021;595(7867):426-431. (In eng). DOI: 10.1038/s41586-021-03696-9.
16. Moriyama S, Adachi Y, Sato T, et al. Temporal maturation of neutralizing antibodies in COVID-19 convalescent individuals improves potency and breadth to circulating SARS-

CoV-2 variants. Immunity 2021;54(8):1841-1852.e4. (In eng). DOI: 10.1016/j.immuni.2021.06.015.
17. Cho A, Muecksch F, Schaefer-Babajew D, et al. Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. Nature 2021 (In eng). DOI: 10.1038/s41586-021-04060-7.
18. Sakharkar M, Rappazzo CG, Wieland-Alter WF, et al. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. Science immunology 2021;6(56) (In eng). DOI: 10.1126/sciimmunol.abg6916.
19. Hurlburt NK, Seydoux E, Wan YH, et al. Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. Nature communications 2020;11(1):5413. (In eng). DOI: 10.1038/s41467-020-19231-9.
20. Ebinger JE, Fert-Bober J, Printsev I, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nature medicine 2021;27(6):981-984. (In eng). DOI: 10.1038/s41591-021-01325-6.
21. Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science (New York, NY) 2021 (In eng). DOI: 10.1126/science.abg9175.