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Article

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve around the world, generating new variants that are of concern based on their potential for altered transmissibility, pathogenicity, and coverage by vaccines and therapeutics[1–5]. Here we report that 20 human sera, drawn 2 or 4 weeks after two doses of BNT162b2, neutralize engineered SARS-CoV-2 with a USA-WA1/2020 genetic background (a virus strain isolated in January 2020) and spike glycoproteins from the newly emerged B.1.617.1, B.1.617.2, B.1.618 (all first identified in India) or B.1.525 (first identified in Nigeria) lineages. Geometric mean plaque reduction neutralization titers against the variant viruses, particularly the B.1.617.1 variant, appear lower than the titer against USA-WA1/2020 virus, but all sera tested neutralize the variant viruses at titers of at least 40. The susceptibility of these newly emerged variants to BNT162b2 vaccine-elicited neutralization supports mass immunization as a central strategy to end the coronavirus disease 2019 (COVID-19) pandemic across geographies.

Since its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused >160 million infections with >3.3 million deaths due to coronavirus disease 2019 (COVID-19) worldwide (https://coronavirus.jhu.edu/). Although coronaviruses have a proofreading mechanism to maintain their long genomic RNAs[6], mutations have continuously emerged in the circulating viruses. Because the viral spike protein (S) binds to angiotensin-converting enzyme 2 (ACE2), the cellular receptor for virus attachment, and mediates membrane fusion during viral entry, mutations in spike can alter SARS-CoV-2 transmission, tissue tropism, and disease outcome[7]. Indeed, the first prevalent spike mutation, D614G, promotes spike binding to ACE2, leading to enhanced SARS-CoV-2 transmission[3,8–11]. Subsequently, another spike mutation, N501Y, emerged convergently in several variants from multiple locations, including the United Kingdom (lineage B1.1.7), Brazil (lineage P.1), and South Africa (lineage B.1.351)[2]. The N501Y mutation also increases the affinity of the spike for ACE2 and increases viral transmission[12,13]. Some mutations in the spike, such as E484K, contribute to evasion of antibody neutralization. The E484K mutation has emerged independently in many variants, such as P.1, B.1.351, B.1.526 (first identified in New York), B.1.525 (first identified in Nigeria), and P3 (first identified in the Philippines)[1,2,14]. Thus, as the COVID-19 pandemic continues, it is critical to closely monitor the impact of new variants on viral transmission, pathogenesis, and vaccine and therapeutic efficacies.

BNT162b2, an mRNA vaccine that expresses the full prefusion spike glycoprotein of SARS-CoV-2, showed an efficacy of 95% against COVID-19[15]. The United States Food and Drug Administration has authorized BNT162b2 for vaccination of individuals 12 years of age and older under emergency use provisions. Although the sequence of BNT162b2 mRNA is based on the original SARS-CoV-2 isolate[16], we and others have shown that sera from those immunized with BNT162b2 retained neutralizing activity against all tested variants, including the B.1.1.7, P.1, B.1.351, B.1.429, B.1.526, and B1.1.7+E484K lineages[1,2,4,5,17]. Since then, a massive second wave of COVID-19 in India has been associated with the expansion of variant B.1.617.1 to 32 countries, B.1.617.2 to 49 countries, and B.1.618 to 6 countries (https://cov-lineages.org/lineages/lineage_B.1.618.html). The B.1.617.2 variant has shown evidence of particularly high transmissibility in the United Kingdom[18]. In addition, variant B.1.525, initially detected in Nigeria, has spread to 49 countries. All these variants currently circulate in the United States. The World Health Organization has designated B.1.617 lineage as a variant of concern and B.1.525 as a variant of interest[18]. This study analyzes BNT162b2-elicited neutralization against these newly emerged variants.

Results

To examine variants' effects on neutralization, we used a reverse genetic system to swap the complete spike gene from different variants into an early SARS-CoV-2 isolate USA-WA1/2020 [defined as wild-type (WT); Extended data Fig. 1a][19]. Five chimeric viruses were prepared: (i) B.1.525-spike with Q52R, A67V, 67/70 deletion (Δ67/70), 145 deletion (Δ145), E484K, D614G, Q677H, and F888L from the B.1.525 variant[18]; (ii) B.1.617.1-spike with G142D, E154K, L452R, E484Q, D614G, P618R, Q1071H, H1101D, and a synonymous mutation at D111 (nucleotide T21895C) from the B.1.617.1 variant; (iii) B.1.617.2-spike with T19R, G142D, L452R, T478K, D614G, P681R, and D950N from an early B.1.617.2 variant (GISAID accession ID: EPI_ISL_1663247); (iv) B.1.617.2-v2-spike

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with the mutations in B.1.617.2-spike plus an additional E156G substitution and F157-R158 deletion (Δ157-158) found in the currently circulating B.1.617.2 isolates[18]; and (v) B.1.618-spike with H49Y, Y145-H146 deletion (Δ145-146), E484K, and D614G from the B.1.618 variant[20]. All mutant viruses yielded infectious titers of $>10^7$ plaque-forming units (PFU)/ml. The B.1.617.1-spike virus formed smaller plaques than other viruses on Vero E6 cells (Extended data Fig. 1b). All viruses were quantified for their viral RNA genome to PFU ratios, a parameter to indicate virus infectivity. None of the variant spikes significantly altered the viral RNA to PFU ratios (Extended data Fig. 1c), suggesting similar specific infectivities of the viral stocks. The complete spikes of all viral stocks were sequenced to ensure no undesired mutations.

To compare the neutralization susceptibility of different variants, we performed 50% plaque reduction neutralization testing ($PRNT_{50}$) using a panel of 20 sera collected from BTN162b2-immunized human subjects from a pivotal clinical trial[15,21]. The serum specimens were drawn 2 or 4 weeks after two immunizations with 30 μg of BNT162b2, spaced three weeks apart (Extended data Fig. 2). Each serum was tested simultaneously for its $PRNT_{50}$ against the WT and mutant viruses (Extended data Table 1). All the sera neutralized the WT and all mutant viruses with titers of 1:40 or higher (Fig. 1). The geometric mean neutralizing titers against the WT, B.1.525-spike, B.1.617.1-spike, B.1.617.2-spike, B.1.617.2-v2-spike, and B.1.618-spike viruses were 502, 320, 157, 355, 343, and 331, respectively (Fig. 1). The results indicate that neutralization of all variants, except the B.1.617.1 variant, was only modestly reduced relative to neutralization of WT virus. Though neutralization of B.1.617.1 was more reduced, BNT162b2 immune sera efficiently neutralized the B.1.617.1 virus and all the other viruses.

Discussion

In response to the global pandemic of COVID-19, the scientific community has increased surveillance to identify mutations in circulating SARS-CoV-2 strains that might increase infectivity, enhance pathogenicity, or alter coverage by therapeutics and vaccines. Such information is essential to guide public policy and countermeasure development. As part of ongoing diligence on coverage of variants by the BNT162b2 vaccine, we have engineered variant spike genes into the backbone of the USA-WA1/2020 isolate, and, using the gold standard $PRNT_{50}$ assay, we have tested neutralization of the resulting viruses by a panel of BTN162b2-immunized human sera drawn 2 or 4 weeks after two doses of BNT162b2 given three weeks apart[4,5]. Among all tested viruses, those with spike proteins from B.1.351[4] and B.1.617.1 (this study) exhibited the greatest reduction in neutralization by the sera, with $PRNT_{50}$'s 0.36 times and 0.31 times, respectively, that of USA-WA1/2020. Similarly, a recent study found that BNT162b2-immune sera neutralized a clinical B.1.617.1 isolate with 0.14 times the neutralization titer of the sera against WT virus[22]. Other studies have found that BNT162b2-immune sera have 0.25 to 0.35 times the inhibitory titer against pseudovirus with a B.1.617.1 spike compared to that against WT spike pseudovirus[23], and that BNT162b2 immune sera inhibit pseudovirus with a B.1.618 spike to 0.37 times the serum inhibition titer against WT spike pseudovirus[20]. Our results showed that among the four tested variants of Indian origin, B.1.617.1 was the least neutralized, probably due to the presence of both L452R and E484Q substitutions at the receptor binding site, potentially under positive selection for resistance to neutralization by antibodies[1,14,24]. Nevertheless, all variants were still neutralized by all tested sera at titers of ≥40. The reduced neutralization could be a combined effect of mutation-mediated escape from antibody binding and mutation-altered spike function.

A recent real-world study in participants who had received two doses of BNT162b2 demonstrated an effectiveness of 75% against any documented infection and 100% against documented severe, critical, or fatal disease caused by the variant B.1.351[25], which showed a similar reduction of neutralization titers as B.1.617.1. Consistent with the modest reduction in neutralization of the B.1.617.2 variants by BNT162b2-elicited sera reported here, a test negative case control study conducted in the United Kingdom found that the real world effectiveness of two doses of BNT162b2 against B.1.617.2 virus was only modestly reduced to 87.9%, compared to 93.4% effectiveness against B.1.1.7 lineage virus[26]. Thus, reductions in neutralization like those observed in this study have not been demonstrated to result in loss of vaccine efficacy against disease. BNT162b2 elicits not only neutralizing antibodies, but also spike-specific $CD4^+$ and $CD8^+$ T cells and non-neutralizing antibody-dependent cytotoxicity, which can also serve as immune effectors[27,28]. Because neutralization titers do not measure all potentially protective vaccine responses, they cannot substitute for studies of vaccine efficacy and real-world effectiveness of COVID-19 vaccines against variants.

A limitation of the current study is the potential for mutations to alter neutralization by affecting spike function rather than antigenicity, even though the variant viruses exhibited similar infectious titers and specific infectivities to the original USA-WA1/2020 isolate. In addition, the study only examined the effect of mutations in the spike glycoproteins. Mutations outside the spike gene could also affect viral replication and host immune response. This study did not examine the durability of neutralization titers against the variant viruses.

New variants will continue to emerge as the pandemic persists. To date, there is no evidence that virus variants have escaped BNT162b2-mediated protection from COVID-19. Therefore, increasing the proportion of the population immunized with current safe and effective authorized vaccines remains a key strategy to minimize the emergence of new variants and end the COVID-19 pandemic.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03693-y.

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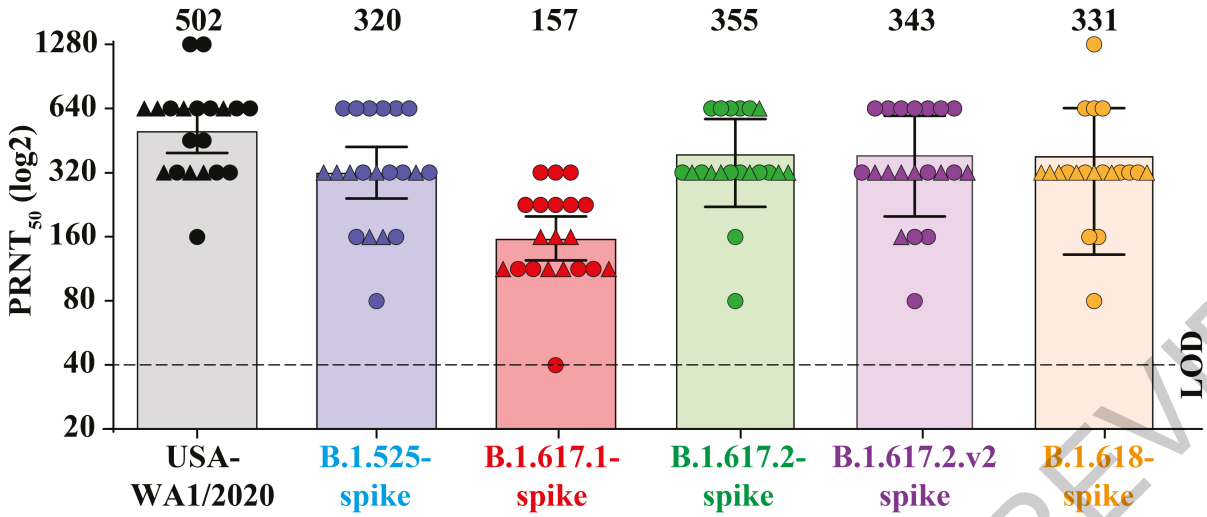

Fig. 1 | Neutralization of USA-WA1/2020 and variant SARS-CoV-2's by BNT162b2-immune sera. Neutralization of variant SARS-CoV-2's by BNT162b2 vaccine-elicited sera. The $PRNT_{50}$ results for USA-WA1/2020 and variant viruses are plotted. Individual $PRNT_{50}$ values are presented in Extended Data Table 1. Each data point represents the geometric mean $PRNT_{50}$ against the indicated virus obtained with a serum sample obtained 2 weeks (circles) or 4 weeks (triangles) after the second dose of vaccine. The $PRNT_{50}$'s were determined in duplicate assays, and the geometric means were calculated (n=20, pooled from two independent experiments). The heights of bars and the numbers over the bars indicate geometric mean titers. The horizontal bars indicate 95% confidence intervals. The dashed line indicates the limit of detection (LOD) at 1:40. Statistical analysis was performed using the two-tailed Wilcoxon matched-pairs signed-rank test. The statistical significance of the difference between geometric mean titers in the USA-WA1/2020 neutralization assay and in each variant virus neutralization assay with the same serum samples are as follows: $P = 0.002$ for B.1.525-spike, $P < 0.0001$ for B.1.617.1-spike, $P = 0.001$ for B.1.617.2-spike, $P = 0.004$ for B.1.617.2-v2-spike, $P = 0.001$ for B.1.618-spike.

Methods

Cells

African green monkey kidney epithelial Vero E6 cells (ATCC, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco/Thermo Fisher, Waltham, MA, USA) with 10% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 1% antibiotic/streptomycin (Gibco). The cell line was performed authentication through STR profiling by ATCC and tested negative for mycoplasma.

Construction of SARS-CoV-2s with variant spikes

All mutations from individual variant spike genes were engineered into an infectious cDNA clone of isolate USA-WA1/2020[19]. The spike mutations were introduced using a standard PCR-based mutagenesis method. A detailed protocol for construction of recombinant SARS-CoV-2 was previously reported[29]. Briefly, the full-length cDNAs of viral genome containing the variant spike mutations were assembled by T4 ligase-mediated *in vitro* ligation. The resulting genome-length cDNAs were used as templates to *in vitro* transcribe full-length viral RNAs. The *in vitro* transcribed full-length viral RNAs were electroporated into Vero E6 cells. When electroporated cells developed cytopathic effects (due to recombinant virus production and replication) on day 2 post electroporation, the original viral stocks (P0) were harvested from culture medium. The P0 viruses were amplified for another round on Vero E6 cells to produce the P1 stocks of viruses. The infectious titers of P1 viruses were measured by plaque assay on Vero E6 cells as previously described[19]. The complete sequences of spike genes from the P1 viruses were verified by Sanger sequencing to ensure no undesired mutations. The P1 viruses were used for subsequent neutralization testing.

Characterization of wild-type and mutant recombinant SARS-CoV-2s

To determine the specific infectivity of each virus, we quantified the P1 stocks for their genomic RNA content and plaque-forming units (PFU) by RT-qPCR and plaque assay on Vero E6 cells, respectively. The protocols for RT-qPCR and plaque assay have been reported previously[3]. Genomic viral RNA to PFU ratios (genomes/PFU) were calculated to indicate the specific infectivity of each virus preparation.

BTN162b2 vaccine-immunized human sera

A panel of 20 serum specimens was collected from 15 BTN162b2-immunized participants in a clinical trial[15,21]. The sera were collected 2 or 4 weeks after two doses of 30 μg BNT162b2 mRNA, spaced 3 weeks apart (Extended data Fig. 2). Five of the 20 participants provided sera at both 2 and 4 weeks after the second dose of vaccine, as detailed in the footnote to Extended data Table 1.

Plaque-reduction neutralization assay

A 50% plaque-reduction neutralization test ($PRNT_{50}$), representing a gold standard of neutralization assay, was performed to quantify serum-mediated virus suppression. Individual sera were 2-fold serially diluted in culture medium with a starting dilution of 1:40. The diluted sera were mixed with 100 PFU of WT USA-WA1/2020 or variant mutant SARS-CoV-2. After 1-h incubation at 37 °C, the serum and virus mixtures were inoculated onto 6-well plates with a monolayer of Vero E6 cells pre-seeded the previous day. The minimal serum dilution that suppressed >50% of viral plaques is defined as $PRNT_{50}$. A detailed $PRNT_{50}$ protocol was reported previously[21,30].

Statistical analysis

Statistical analyses were performed by Graphpad Prism 9 for all experiments as detailed in legends to individual figures.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Source data for generating main figures are available in the online version of the paper. Any other information is available upon request.

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Author contributions Conceptualization, K.U.J, U.S., X.X., K.A.S., A.M., P.R.D., P.-Y.S.; Methodology, J.L., Y.L., H.X., J.Z., S.C.W., K.A.S., H.C., A.M., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; Investigation, J.L., Y.L., H.X., J.Z., S.C.W., K.A.S., H.C., M.C., D.C., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; Data Curation, J.L., Y.L., M.C., D.C., X.X., P.-Y.S.; Writing-Original Draft, J.L., Y.L., U.S., X.X., P.R.D., P.-Y.S.; Writing-Review & Editing, S.C.W., K.A.S., A.M., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; Supervision, K.U.J., U.S., X.X., P.R.D., P.-Y.S.; Funding Acquisition, K.U.J., U.S., P.R.D., P.-Y.S.

Competing interests X.X. and P.-Y.S. have filed a patent on the reverse genetic system of SARS-CoV-2. K.A.S., H.C., M.C., D.C., K.U.J., and P.R.D. are employees of Pfizer and may hold stock options. A.M. and U.S. are employees of BioNTech and may hold stock options. Y.L., H.X., J.Z., X.X., and P.-Y.S. received compensation from Pfizer to perform the project.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-021-03693-y.

Correspondence and requests for materials should be addressed to U.S., X.X., P.R.D. or P.-Y.S.

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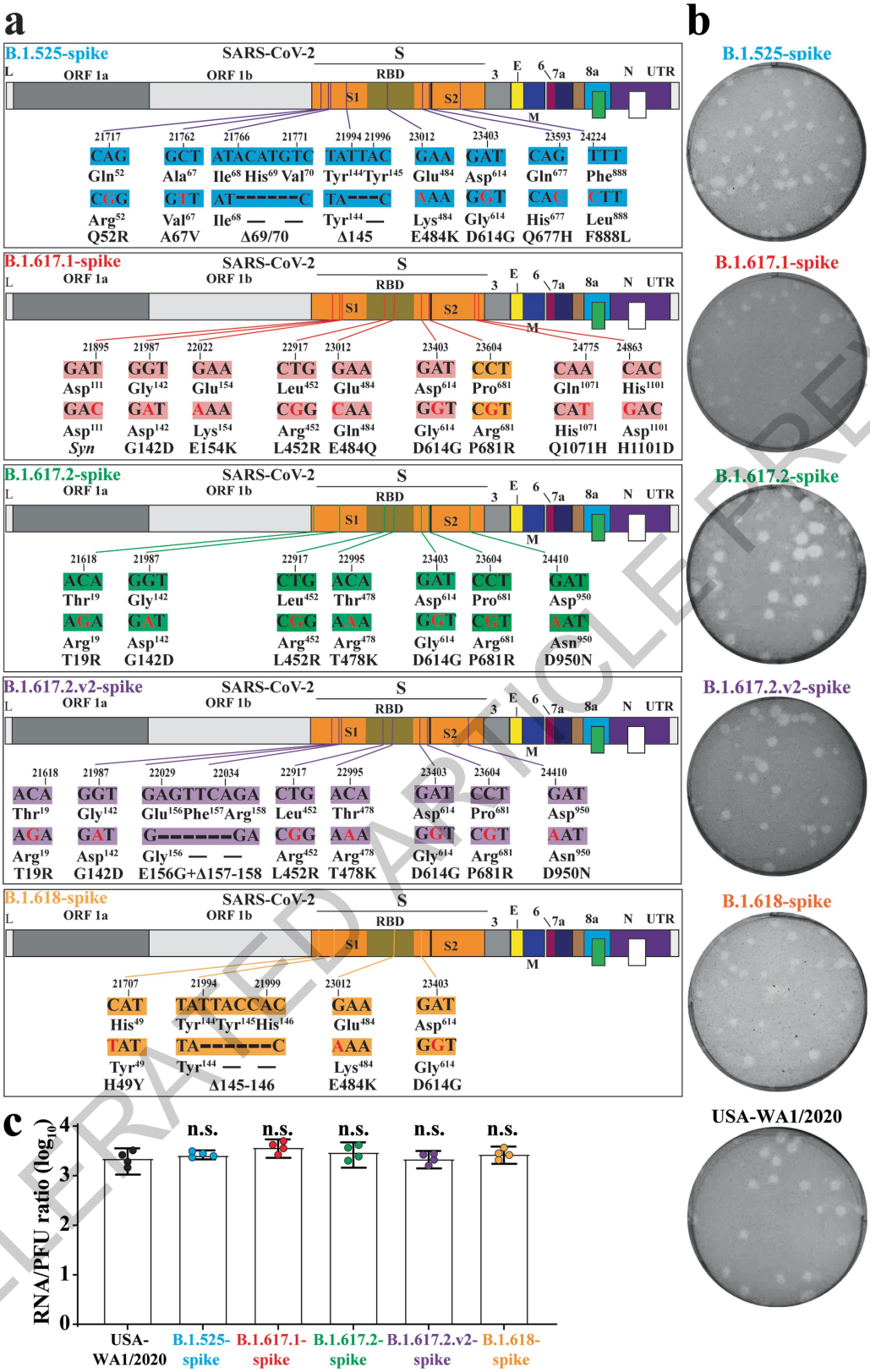
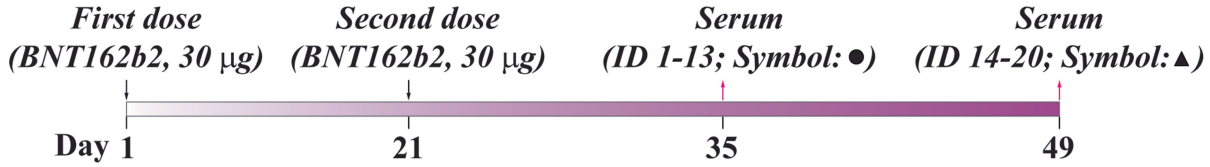


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Construction and characterization of SARS-CoV-2s with variant spikes. a, Diagram of engineered variant spike mutations. Mutations from variant spikes were engineered into isolate USA-WA1/2020. Mutations and deletions are indicated in red and by dotted lines, respectively. Nucleotide and amino acid positions are also indicated. Different regions of SARS-CoV-2 genome are indicated: L (leader sequence), ORF (open reading frame), RBD (receptor binding domain), S (spike glycoprotein), S1 (N-terminal furin cleavage fragment of S), S2 (C-terminal furin cleavage fragment of S), E (envelope protein), M (membrane protein), N (nucleoprotein), and UTR (non-translated region). **b**, Plaque morphologies of recombinant SARS-CoV-2s. Plaque assays were performed on Vero E6 cells in 6-well plates. **c**, Comparison of viral genomic RNA versus plaque-forming unit ratios (genomes/PFU) of recombinant SARS-CoV-2's. The genomic RNA and PFU of individual virus stocks were measured by RT-qPCR and plaque assay, respectively. The genomes/PFU ratios were calculated to determine specific infectivities. Dots represent individual biological replicates from 4 aliquots of viruses (n=4, one experiment). The values in the graph represent means with 95% confidence intervals. A non-parametric two- tailed Mann-Whitney test was used to determine significant differences between USA-WA1/2020 and variant viruses. *P* values were adjusted using the Bonferroni correction to account for multiple comparisons. Differences were considered significant if $P < 0.05$; n.s., no statistical difference.



Extended Data Fig. 2 | BNT162b2 immunization scheme and serum collection. Twenty human sera were obtained from 15 trial participants at 2 weeks (circles) or 4 weeks (triangles) after the second dose of BNT162b2 vaccine. Five of the 15 participants provided sera at both 2 and 4 weeks after the second dose of vaccine.

Extended Data Table 1 | $PRNT_{50}$ values of sera from BNT162b2-immunized trial participant against USA-WA1/2020 and variant SARS-CoV-2

Serum*				$PRNT_{50}$†									
ID‡	Age (years)	Sex	Week	USA-WA1/2020			B.1.525-spike	B.1.617.1-spike			B.1.617.2-spike	B.1.617.2-v2-spike	B.1.618-spike
				Exp1	Exp2	GMT		Exp1	Exp2	GMT			
1	68	F	2	640	640	640	640	320	320	320	320	320	320
2	67	M	2	160	160	160	80	40	40	40	80	80	80
3	68	F	2	1280	1280	1280	640	320	320	320	640	640	1280
4	66	F	2	320	320	320	320	80	160	113	320	160	160
5	30	M	2	320	640	453	160	80	160	113	320	320	160
6	23	F	2	320	320	320	320	80	160	113	160	160	320
7	54	M	2	640	640	640	640	160	320	226	640	640	640
8	69	F	2	320	320	320	160	80	160	113	320	320	320
9	65	M	2	640	640	640	640	160	320	226	640	640	640
10	38	F	2	640	640	640	640	320	320	320	640	640	640
11	44	F	2	320	640	453	640	160	320	226	320	640	320
12	52	F	2	640	640	640	320	160	320	226	320	640	320
13	28	M	2	1280	1280	1280	320	160	320	226	640	640	320
14	69	F	4	320	320	320	160	80	160	113	320	320	320
15	68	F	4	320	320	320	160	80	160	113	320	320	320
16	26	F	4	320	320	320	320	80	160	113	320	320	320
17	54	M	4	640	640	640	320	160	160	160	640	320	320
18	35	F	4	640	640	640	320	160	160	160	320	320	320
19	44	F	4	640	640	640	320	80	160	113	320	320	320
20	52	F	4	640	640	640	320	160	160	160	320	160	320
		GMT§		485	520	502	320	126	197	157	355	343	331
		95% CI‡		380-619	410-659	397-636	242-423	96-163	155-245	124-199	278-452	260-452	253-433

*Pairs of sera were obtained from five of the twenty participants at both 2 and 4 weeks after the second dose of vaccine. The paired sera have ID' 1 and 15, 7 and 17, 8 and 14, 11 and 19, and 12 and 20.
†The data for USA-WA1/2020 and B.1.617.1 are from two independent experiments. The results for other variants are from one experiment each. For each independent experiment, the individual $PRNT_{50}$ value is the geometric mean of duplicate plaque assay results; no differences were observed between the duplicate assays.
‡The serum donors were White, except for donor 10, who was Asian. All donors were of non-Hispanic/non-Latino ethnicity.
§Geometric mean neutralizing titers.
‡95% confidence interval (95% CI) for the GMT.

nature research

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No code and software used for the data collection
Data analysis	Graphpad Prism 9

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Extended Data and source data for generating main figures are available in the online version of the paper. Any other information is available upon request.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. Sample size was chosen based on previous experience and availability, 20 samples were collected from BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial (ClinicalTrials.gov identifier: NCT04368728). Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.).
Data exclusions	No data was excluded in the study.
Replication	The experiments were performed twice with 20 different samples. The averaged results from the duplication were reported in this study. All attempts at replication were successful.
Randomization	No randomization was performed. All samples were analyzed for the neutralizing activities against WT SARS-CoV-2 and variants in the same experimental settings.
Blinding	Patient information was blinded in the study. Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.). The investigators were not blinded to the allocation during the experiments or to the outcome assessment. Blinding is not necessary because the results are quantitative and did not require subjective judgment or interpretation. Blinding is not typically used in the field.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 cells (ATCC® CRL-1586) were obtained from ATCC
Authentication	ATCC have comprehensively performed authentication on cell lines through STR profiling.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.