Vaccination with SARS-CoV-2 variants of concern protects mice from challenge with wild-type virus

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Abstract

Vaccines against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) have been highly efficient in protecting against Coronavirus Disease 2019 (COVID-19). However, the emergence of viral variants that are more transmissible and, in some cases, escape from neutralizing antibody responses has raised concerns. Here, we evaluated recombinant protein spike antigens derived from wild-type SARS-CoV-2 and from variants B.1.1.7, B.1.351, and P.1 for their immunogenicity and protective effect in vivo against challenge with wild-type SARS-CoV-2 in the mouse model. All proteins induced high neutralizing antibody responses against the respective viruses but also induced high cross-neutralizing antibody responses. The decline in neutralizing titers between variants was moderate, with B.1.1.7-vaccinated animals having a maximum fold reduction of 4.8 against B.1.351 virus. P.1 induced the most cross-reactive antibody responses but was also the least immunogenic in terms of homologous neutralization titers. However, all antigens protected from challenge with wild-type SARS-CoV-2 in a mouse model.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan, China. Since then, the virus has caused the Coronavirus Disease 2019 (COVID-19)
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**Competing interests:** I have read the journal's policy and the authors of this manuscript have the following competing interests: The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Fatima Amanat is also listed on the serological assay patent application as co-inventors. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

**Abbreviations:** AUC, area under the curve; BSL-3, biosafety level 3; COVID-19, Coronavirus Disease 2019; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; hACE2, human angiotensin converting enzyme 2; HRP, horseradish peroxidase; MEM, minimal essential medium; NTA, nitrilotriacetic acid; NTD, N-terminal domain; RBD, receptor binding domain; RT, room temperature; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; TCID₅₀, 50% tissue culture infectious doses; VoC, variant of concern.

pandemic leading to approximately 5 million official deaths globally (as of November 2021). While coronaviruses usually mutate more slowly than other RNA viruses due to the proof-reading activity of their replication machinery [1], viral variants started to emerge in the summer of 2020 in humans and mink in Europe [2–4]. In late 2020, additional variants, termed variants of concern (VoCs) emerged in the United Kingdom [5], in South Africa [6], and in Brazil [7]. These variants, B.1.1.7, B.1.351, and P.1, are more infectious than wild-type SARS-CoV-2 and feature extensive changes in both the receptor binding domain (RBD) and the N-terminal domain (NTD) of the spike protein. These 2 domains harbor the vast majority of neutralizing epitopes [8–14], and, consequently, it has been observed that—especially for B.1.351—the neutralizing activity of wild-type postinfection and postvaccination sera is reduced [15–18]. In addition, efficacy and effectiveness of vaccines against B.1.351 have been shown to be somewhat reduced, depending on the type of vaccine platform used [19,20]. For one currently licensed vaccine, the efficacy against B.1.351 was lost [21]. Updated vaccines based on variant spike sequences are currently being tested by vaccine producers and may be licensed in the future for variants that escape vaccine-induced immunity to an even larger degree, e.g. the B.1.1.529 (Omicron) variant. However, the process of updating vaccine antigens to match circulating variants is not as straightforward as it seems. Several variants might circulate simultaneously, making it difficult to choose the right antigen for optimal protection. Of course, multivalent vaccines that include more than one variant antigen can be formulated, but this increases complexity and decreases the amount of vaccine doses that can be manufactured. Understanding the antigenic relationship between variants is therefore of high importance.

Here, we vaccinated mice with recombinant spike proteins from the wild-type Wuhan-1 strain, B.1.1.7, B.1.351, and P.1 and assessed the resulting cross-neutralization in the sera. Furthermore, we challenged the animals with a wild-type strain (SARS-CoV-2/human/USA/USA-WA1/2020 (WA1)) of SARS-CoV-2 to determine if variant vaccine antigens would still protect from the prototypic virus. Adjuvanted, recombinant spike proteins were chosen as antigen since they reflect vaccines currently in clinical development by Novavax, Sanofi Pasteur, and other vaccine manufacturers.

**Results**

**Variant spike proteins induce cross-neutralizing antibodies in the mouse model**

First, we vaccinated BALB/c mice twice with adjuvanted recombinant spike proteins of wild-type SARS-CoV-2 (Wuhan-1), B.1.1.7, B.1.351, and P.1. Three weeks postboost, the animals were bled and the neutralizing activity of their serum was assessed in a well-established microneutralization assay with authentic SARS-CoV-2 [22]. When tested against the respective virus from which the vaccine antigen was derived, all animals mounted strong neutralizing antibody responses (Fig 1A; geometric mean neutralization titers of 6,924, 6,673, 4,690, and 3,246 for wild type, B.1.1.7, B.1.351, and P.1, respectively), while negative controls showed no neutralizing activity (Fig 1B). The negative control group received an irrelevant control protein, influenza virus hemagglutinin. However, there was a trend toward B.1.1.7-vaccinated animals showing higher neutralizing capacity against homologous virus as compared to the other spike variant antigens. P.1 seemed to induce the lowest neutralizing activity against homologous viruses. These differences were small and only significant for B.1.1.7 versus P.1.

As expected, when testing for cross-reactivity, the different spike proteins induced the highest neutralization titers against the homologous viruses. Sera from wild-type spike-vaccinated animals neutralized WA1 best, followed by B.1.1.7, P.1, and B.1.351 (Fig 1C; geometric mean neutralization titers of 6,924, 4,994, 2,071, and 2,291 for wild type, B.1.1.7, B.1.351, and P.1,
Crossreactivity among SARS-CoV-2 variants in mice

A. Neutralization of homologous strain

B. Negative controls

C. Wuhan1 (wild type) immunized

D. B.1.1.7 immunized

E. B.1.351 immunized

F. P.1 immunized
respectively). Sera from B.1.1.7 vaccinated animals neutralized B.1.1.7 best, followed by wild type, P.1, and B.1.351 (Fig 1D; geometric mean neutralization titers of 3,207, 6,673, 1,381, and 1,518 for wild type, B.1.1.7, B.1.351, and P.1, respectively). For B.1.351-vaccinated animals, we detected the highest titers against B.1.351 followed by wild type, B.1.1.7, and P.1 (Fig 1E; geometric mean neutralization titers of 1,580, 1,458, 4,690, and 1,131 for wild type, B.1.1.7, B.1.351, and P.1, respectively). P.1 induced a surprisingly uniform level of immunity with the lowest drop to wild-type virus followed by B.1.351 and B.1.1.7 (Fig 1F; geometric mean neutralization titers of 2,235, 1,276, 1,460, and 3,246 for wild type, B.1.1.7, B.1.351, and P.1, respectively). The steepest drops in neutralization were detected for B.1.1.7 to B.1.351 (4.8-fold), from B.1.1.7 to P.1 (4.4-fold), and from B.1.351 to P.1 (4.2-fold). Importantly, we did not observe complete loss in neutralizing activity against any of the viruses.

We used antigenic cartography [23] to visualize the antigenic relationships between the tested viruses and sera (Fig 2). The B.1.351 virus is positioned furthest from the WA1 virus, and P.1 and B.1.1.7 are approximately equal distance from WA1 in opposite directions. The sera loosely cluster in the vicinity of the antigen they were raised against.

**Antibody binding is less affected than neutralization**

We repeated our analysis using an enzyme-linked immunosorbent assay (ELISA) with the respective spike proteins as substrates. While neutralization requires binding of antibodies to a limited number of epitopes mostly on RBD and NTD, many more binding epitopes exist on the spike protein [8]. Therefore, more even reactivity was expected. We did detect differences in reactivity when binding was tested against the respective matched spikes (Fig 3A; geometric mean area under the curve (AUC) values of 13,328, 10,317, 20,086, and 11,373 for wild type, B.1.1.7, B.1.351, and P.1, respectively), but while these differences were statistically significant in 3 cases, they were relatively small. However, it seemed that vaccination with B.1.351 induced slightly more homologous binding antibodies compared to the other immunogens. Low background reactivity was detected in sera of the control animals (Fig 3B).

Both wild-type spike and B.1.1.7 spike induced relatively even binding antibody responses (Fig 3C and 3D; wild type: geometric mean AUCs of 13,328, 11,545, 13,942, and 12,513 for wild type, B.1.1.7, B.1.351, and P.1, respectively; B.1.1.7: geometric mean AUCs of 9,237, 10,317, 10,765, and 7,807 for wild type, B.1.1.7, B.1.351, and P.1, respectively) with a maximum fold reduction of 1.2- and 1.3-fold, respectively. A stronger reduction was detected when B.1.351 was used as immunogen with 3.2-fold and 3.8-fold reduction in binding to wild-type and B.1.1.7 spike, respectively (Fig 3E; geometric mean AUCs of 6,352, 5,535, 20,086, and 6,990 for wild type, B.1.1.7, B.1.351, and P.1, respectively). The drop for P.1 was smaller (2.9-fold). P.1 also induced comparable binding antibody response with a maximum fold reduction of 1.5-fold against B.1.1.7 (Fig 3F; geometric mean AUCs of 9,811, 7,377, 11,437, and 11,373 for wild type, B.1.1.7, B.1.351, and P.1, respectively).
These discrepancies between neutralization and binding antibody profiles allowed us to calculate ratios between binding and neutralizing antibodies. The best (higher) ratios (indicating a higher proportion of neutralizing antibodies) were found in sera from wild-type and B.1.1.7-vaccinated mice (S1 Fig). For each vaccination group, the ratio was always best against the homologous virus and dropped with antigenic distance (S1 Fig). The most stable ratio was observed for P.1-vaccinated animals (S1 Fig).

All spike-vaccinated animals are protected against challenge with wild-type SARS-CoV-2

Finally, we wanted to assess if the induced neutralizing antibody responses can protect animals from challenge with prototypic SARS-CoV-2 strain WA1. Since BALB/c mice are not susceptible to this virus, they had to be presensitized via intranasal transduction with adenovirus expressing human angiotensin converting enzyme 2 (hACE2) before challenge, as previously described. The main readout for the challenge experiment were virus titers in the lungs of infected animals. On day 2 postchallenge, control animals showed high viral loads in their lungs (approximately 10^6 plaque forming units per ml of lung homogenate) (Fig 4A). In contrast, no virus was detected in wild-type and P.1 spike-vaccinated animals. For B.1.1.7 and B.1.351 spike-vaccinated animals, one animal per group showed traces of virus replication in the lung, but titers were barely above the limit of detection. On day 5 postinfection, no virus
was detectable in the lungs of vaccinated individuals, while control animals still showed high virus loads (Fig 4B).

Discussion

The emergence of SARS-CoV-2 variants is concerning both in terms of infection control (because many variants are more infectious), as well as in terms of vaccine effectiveness, due to the potential for immune escape. While several vaccines, which are either in clinical development or already in use, show good efficacy or effectiveness against most variants [19,20,24,25],

Fig 3. All vaccinated groups have cross-reactive antibodies in their sera against spike proteins of wild type, B.1.1.7, B.1.351, and P.1. (A) An ELISA was performed using sera from each group and tested for binding with the homologous spike protein, and the binding of each group against the respective spike protein is represented as AUC. (B) Binding of the samples in the negative control group was also tested against the spike proteins of wild-type SARS-CoV-2, B.1.1.7, B.1.351, and P.1 isolates. (C-F) Sera from mice vaccinated with wild-type spike protein (C), B.1.1.7 spike protein (D), B.1.351 spike protein (E), and P.1 spike protein (F) were tested against the spike proteins of wild type, B.1.1.7, B.1.351, and P.1. Binding is shown as AUC, and the differences in binding are indicated by horizontal bars with the calculated fold increase or decrease. Statistical significance was tested with an ANOVA corrected for multiple comparisons. P values are shown for comparisons that resulted in statistical significance. Underlying raw data can be found in the S1 Data. AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

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Fig 4. Vaccination with spike proteins of B.1.1.7, B.1.351, and P.1 protects against challenge with wild-type SARS-CoV-2 in a mouse model. (A, B) All groups of vaccinated mice were challenged with authentic SARS-CoV-2 after sensitization with AdV-hACE2 five days prior to infection. After infection, viral loads in the lungs were quantified via a plaque assay on day 2 (A) and day 5 (B). Underlying raw data can be found in the S1 Data. AdV-hACE2, adenovirus-human angiotensin converting enzyme 2; PFU, plaque-forming unit; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; wt, wild type.

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the vaccine antigens may need to be updated at some point to cover variants that evade neutralizing antibodies more efficiently, like B.1.1.529 (Omicron). However, if variants cocirculate, it will be difficult to select the “right” variant that induces good immune responses across the board. Here, we have tested the cross-neutralization activity of wild type, B.1.1.7, B.1.351, and P.1 adjuvanted protein vaccines in the mouse model. We found that P.1 induces the most balanced immune response across the four tested antigens, supported by four sera raised against P.1 positioned centrally in the antigenic map between WA1, B.1.351, and P.1. Interestingly, while B.1.351 and P.1 share 2 of their RBD mutations and have a mutated residue at position 417 in common (although to different amino acids), a sharp drop in neutralizing activity from B.1.351 to P.1 was observed. However, this relationship was asymmetric, since the drop from P.1 to B.1.351 was much smaller. When considering the results from this study, P.1 should likely be the chosen immunogen for updated vaccines in our mouse model. Our mouse data are similar compared to human cross-neutralization data with the same four variants published by Liu and colleagues [26]. This suggests that mice may be a good model system to study antigenic variability among variants. Such model systems are of importance, as they ensure the continued availability of first-infection sera for the characterization of novel variants. However, there may be subtle differences between mouse strains and certainly between mice and humans, which need to be further explored. Interestingly, binding was much less affected than neutralization. There could be several reasons for that. One reason could be reduced antibody affinity that still allows binding but does not support neutralization anymore. Another reason could be that only a small number of epitopes are targeted by neutralizing antibodies while many more epitopes exist (especially outside the RBD and NTD) that are bound by nonneutralizing antibodies. If some of the neutralizing antibodies lose binding, the effect on overall binding is small because so many nonneutralizing antibodies still bind different epitopes on the very large spike. Similar findings have recently been reported in humans [27]. Importantly, all spike antigens, independently of the lineage, provided robust protection against challenge with the prototypic WA1 strains, suggesting that “updated” vaccines—especially if they induce high neutralizing antibody titers—would sufficiently protect against most other circulating variants as well as the prototypic SARS-CoV-2 strain. Of note, we do not think that the low virus titer (barely above the limit of detection) in one of the B.1.1.7 and in one of the B.1.351-vaccinated animals changes this conclusion. While challenge with variant viruses was not possible at this point in time in the selected mouse system (some variants bind to mouse ACE2 also, making comparisons complicated), we plan to evaluate cross-protection in more detail in the hamster model in the future. However, our work addresses the very pertinent question if a variant-based vaccine (as being developed by several vaccine producers) would protect against a mismatched/heterologous virus.

Our work here has focused on neutralizing and binding antibodies, which have been implicated as correlates of protection for SARS-CoV-2 vaccine-induced immunity [28,29], and reduction in neutralizing antibodies in sera from convalescent individuals and vaccinees against variants has been observed. However, T-cell responses very likely contribute to protection from COVID-19 as well. We have not analyzed T-cell responses in our experimental animals, but others have shown that the impact of variants on these responses is minimal [30]. Another caveat of our study is that we were not able to include B.1.617.2, B.1.617.1, C.37, and B.1.1.529 in our analysis even though these are currently important variants.

In summary, we found that neutralizing titers are always highest against the homologous virus but that antigenic relationships are not necessarily symmetric and that some variant spike proteins induced more balanced responses (e.g., P.1) than others (B.1.351 and B.1.1.7). In addition, the drop in binding antibody is much lower than the drop in neutralizing activity. Nonneutralizing binding antibodies have been shown to play an important role in protection...
for other diseases caused by virus infections including Ebola virus disease and influenza A and B viruses [31–34]. The maintenance of binding antibody and T-cell responses against variants could partially explain the maintenance of vaccine effectiveness, despite the occasional steep drops in neutralizing antibody titers.

**Materials and methods**

**Recombinant proteins**

All recombinant proteins were expressed and purified using Expi293F cells (Life Technologies, Carlsbad, CA), as described in detail previously [22,35]. The spike gene of each respective variant (EPI-ISL_703454, EPI-ISL_745160) was cloned into the pCAGGS vector and used to transfect cells. The cleavage site was deleted by removing the arginine residues, and prolines were added to position 986 and 987 to stabilize the spike trimer. The supernatant was clarified on day 4 posttransfection via centrifugation at 4,000 g for 20 minutes. Ni²⁺-nitrilotriacetic acid (NTA) agarose (Qiagen, Hilden, Germany) was used to purify the protein, as described before [36,37]. EPI-ISL_792680 was cloned into pcDNA3.4 for transient transfection. The endogenous leader peptide was replaced with the tPA secretion signal, 8XHIS and AviTag epitopes were appended, and the substitutions noted above introduced. The spike trimer was expressed by transient transfection in 293F cells and purified by affinity chromatography as previously described [38].

The proteins used for ELISA were purchased from Sino Biological and include the following: 40589-V08B6, 40589-V08B7, 40589-V08B8, and 40589-V08B8, and 40589-V08B1.

**Cells and viruses**

Vero.E6 cells (ATCC CRL-1586, clone E6) were kept in culture using Dulbecco’s modified Eagle medium (Gibco, Waltham, MA), which was supplemented with 10 mL of Antibiotic-Antimycotic (100 U/ml penicillin–100 μg/ml streptomycin–0.25 μg/ml amphotericin B; Gibco), 10% of fetal bovine serum (FBS; Corning, Corning, NY), and 1% HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid; Gibco). Wild-type SARS-CoV-2 (isolate USA-WA1/2020), hCoV-19/South Africa/KRISP-K005325/2020 (B.1.351, BEI Resources NR-54009), hCoV-19/Japan/TY7–503/2021 (P.1, BEI resources NR-54982), and hCoV-19/England/204820464/2020 (B.1.1.7, BEI Resources NR54000) were cultured in Vero.E6 cells for 3 days at 37˚C and then the supernatant was clarified via centrifugation at 1,000 g for 10 minutes. Virus stocks were stored at −80˚C. The protocol was described in greater detail previously [22,39]. All work with authentic SARS-CoV-2 was performed in a biosafety level 3 (BSL-3) facility following institutional guidelines.

**In vivo mouse studies**

All animal procedures were performed by adhering to the Institutional Animal Care and Use Committee (IACUC) guidelines of the Icahn School of Medicine at Mount Sinai IACUC and according to an approved protocol (IACUC-2014-0255). Research was conducted in concordance with the Animal Act PL99-158 (as amended). Six- to 8-week-old female, BALB/c mice were vaccinated via the intramuscular route with 3 μg of each respective protein with 1:1 mixture of AddaVax (Invivogen, San Diego, CA) in a total volume of 50 μL. After 3 weeks, mice were bled and vaccinated again. Three weeks later, mice were administered anesthesia via the intraperitoneal route and then intranasally transduced with AdV-hACE2 at 2.5 × 10⁶ plaque-forming units (PFUs) per mouse. Anesthesia was prepared using 0.15 mg/kg of body weight ketamine and 0.03 mg/kg xylazine in water. Five days later, all mice were infected with wild-type SARS-CoV-2 intranasally with 1 × 10⁵ PFU. Mice were humanely sacrificed on day 2 and
day 5 for assessment of virus in the lungs. Lungs were homogenized using special tubes and a BeadBlaster 24 (Benchmark, Sayreville, NJ) homogenizer [40,41]. Viral load in the lung was quantified via a classic plaque assay [42].

**ELISA**

Ninety-six-well plates (Immulon 4 HBX; Thermo Fisher Scientific, Waltham, MA) were coated with 2 μg/mL of each respective protein with 50 μL/well overnight at 4˚C. The next morning, the coating solution was discarded, and each plate was blocked with 100 μL/well of 3% nonfat milk (AmericanBio; catalog no. AB10109-01000) in phosphate buffered saline containing 0.01% Tween (PBS-T). Blocking solution was kept on the plates for 1 hour at room temperature (RT). Serum samples were tested starting at a dilution of 1:50 with 1:5-fold subsequent serial dilutions. Serum samples were added to the plates for 2 hours at RT. Next, the plates were vigorously washed 3 times with 200 μL/well of PBS-T. Anti-mouse IgG-horseradish peroxidase (HRP)-conjugated antibody (Rockland; catalog no. 610–4302) was used at a dilution of 1:3,000 in 1% nonfat milk in PBS-T, and 100 μL of this solution was added to each well for 1 hour at RT. The plates were washed 3 times with 200 μL/well of PBS-T and dried on paper towels. Developing solution was prepared in sterile water (WFI; Gibco) using SigmaFast OPD (o-phenylenediamine dihydrochloride, catalog no. P9187; Sigma-Aldrich), and 100 μL was added to each well for a total of 10 minutes. To stop the reaction, 50 μL/well of 3 M hydrochloric acid was added, and the plates were read in a plate reader, Synergy 4 (BioTek, Winooski, VT), at an absorbance of 490 nanometers. Data were analyzed in GraphPad Prism 7.

**Neutralization assay**

Twenty-thousand Vero.E6 cells were seeded per well in a 96-well cell culture plate (Corning; 3340) 1 day prior to performing the assay. Serum samples were heat inactivated at 56˚C for 1 hour prior to use. Serum dilutions were prepared in 1× minimal essential medium (MEM; Gibco) supplemented with 1% FBS. Each virus was diluted to 10,000 50% tissue culture infectious doses (TCID$_{50}$s)/mL, and 80 μL of virus and 80 μL of serum were incubated together for 1 hour at RT. After the incubation, 120 μL of virus–serum mixture was used to infect cells for 1 hour at 37˚C. Next, the virus–serum mix was removed and 100 μL of each corresponding dilution was added to each well. A volume of 100 μL of 1X MEM were also added to the plates to get to a total volume of 200 μL in each well. The cells were incubated at 37˚C for 3 days and then fixed with 10% paraformaldehyde (Polysciences, Warrington, PA) for 24 hours. The next day, cells were stained using a rabbit anti-nucleoprotein antibody (Invitrogen; PA5-81794) as primary antibody and a goat anti-rabbit secondary antibody conjugated to HRP (Invitrogen; 31460). This protocol was adapted from an earlier established protocol [22,35,43].

**Antigenic cartography**

A target distance from a serum to each virus is derived by calculating the difference between the logarithm (log$_2$) reciprocal neutralization titer for that particular virus and the log$_2$ reciprocal maximum titer achieved by that serum (against any virus). Thus, the higher the reciprocal titer, the shorter the target distance. As the log$_2$ of the reciprocal titer is used, a 2-fold change in titer will equate to a fixed change in target distance whatever the magnitude of the actual titers. Antigenic cartography [23] was then used to optimize the positions of the viruses and sera relative to each other on a map, minimizing the sum-squared error between map distance and target distance. Each virus is therefore positioned by multiple sera, and the sera themselves are also positioned only by their distances to the viruses. Hence, sera with different neutralization profiles to the virus panel are in separate locations on the map but contribute equally to...
positioning of the viruses. The antigenic cartography software used was written by Sam Wilks and is available as free and open-source software from https://www.antigenic-cartography.org.

Supporting information

S1 Fig. Neutralization over binding ratio varies for each group. (A) The neutralization over binding ratio was calculated and depicted for each group against the homologous virus and homologous spike protein. Statistical analysis was performed with an ANOVA corrected for multiple comparisons, and the p-values are indicated when statistical significance was present. (B-E) Neutralization over binding ratios are shown for groups vaccinated with wild-type spike protein (B), B.1.1.7 spike protein (C), B.1.351 spike protein (D), and P.1 spike protein (E). Underlying raw data can be found in the S1 Data.

S1 Data. This file contains all raw data underlying the figures.

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References

1. Denison MR, Graham RL, Donaldson EF, Eckerle LD, Baric RS. Coronaviruses: an RNA proofreading machine regulates replication fidelity and diversity. RNA Biol. 2011; 8(2):270–9. Epub 2011 Mar 1. https://doi.org/10.4161/rna.8.2.15013 PMID: 21593585; PubMed Central PMCID: PMC3127101.
2. Larsen HD, Fonager J, Lomholt FK, Dalby T, Benedetti G, Kristensen B, et al. Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated with community spread, Denmark, June
to November 2020. Euro Surveill. 2021; 26(5). https://doi.org/10.2807/1560-7917.ES.2021.26.5.210009 PMID: 33541485; PubMed Central PMCID: PMC7863232.

3. Thomson EC, Rosen LE, Shepherd JG, Spreafico R, da Silva Filipe A, Wojczechowsky JA, et al. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. Cell. 2021. Epub 2021 Jan 28. https://doi.org/10.1016/j.cell.2021.01.037 PMID: 33621484; PubMed Central PMCID: PMC7843029.

4. Korber B, Fischer WM, Gnanakaran S, Hopkanson LS, Muller BS, Klima SE, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020; 182(4):812–27.e19. Epub 2020 Jul 3. https://doi.org/10.1016/j.cell.2020.06.043 PMID: 32697968; PubMed Central PMCID: PMC7332439.

5. PHE. Investigation of novel SARS-CoV-2 variant Variant of Concern 202012/01 Technical briefing 5. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/959426/Variant_of_Concern_VOC_202012_01_Technical_Briefing_5.pdf2021.

6. Tegally H, Wilkinson E, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature. 2021; 592(7854):438–43. Epub 2021 Mar 9. https://doi.org/10.1038/s41586-021-03402-9 PMID: 33692065.

7. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science. 2021; 372(6544):815–21. Epub 2021 Apr 14. https://doi.org/10.1126/science.abi2644 PMID: 33853970.

8. Amanat F, Thapa M, Lei T, Ahmed SMS, Adelsberg DC, Carreño JM, et al. SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. Cell. 2021. Epub 2021 Jun 8. https://doi.org/10.1016/j.cell.2021.06.005 PMID: 34192529; PubMed Central PMCID: PMC8185186.

9. McCallum M, De Marco A, Lemp FF, Tortorici MA, Pinto D, Walls AC, et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell. 2021. Epub 2021 Mar 16. https://doi.org/10.1016/j.cell.2021.03.028 PMID: 33761326; PubMed Central PMCID: PMC7962585.

10. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, et al. A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. Science. 2020; 369(6504):650–5. Epub 2020 Jun 22. https://doi.org/10.1126/science.abc6952 PMID: 32571838; PubMed Central PMCID: PMC7319273.

11. Greaney AJ, Starr TN, Gilchuk P, Zost SJ, Binshtein E, Loes AN, et al. Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. Cell Host Microbe. 2021; 29(1):44–57.e9. Epub 2020 Nov 19. https://doi.org/10.1016/j.chom.2020.11.007 PMID: 33259786; PubMed Central PMCID: PMC7676316.

12. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature. 2020; 584(7821):437–42. Epub 2020 Jun 18. https://doi.org/10.1038/s41586-020-2456-9 PMID: 32555388; PubMed Central PMCID: PMC77442695.

13. Alsoussi WB, Turner JS, Case JB, Zhao H, Schmitz AJ, Zhou JQ, et al. A Potently Neutralizing Antibody Protects Mice against SARS-CoV-2 Infection. J Immunol. 2020. Epub 2020 Jun 26. https://doi.org/10.4049/jimmunol.2000583 PMID: 32591393.

14. Turner JS O’Halloran JA, Kalaidina E, Kim W, Schmitz AJ, Zhou JQ, et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. Nature. 2021. Epub 2021 Jun 28. https://doi.org/10.1038/s41586-021-03738-2 PMID: 34182589.

15. Cele S, Gazy I, Jackson L, Hwa S-H, Tegally H, Lustig G, et al. Escape of SARS-CoV-2 501Y.V2 variants from neutralization by convalescent plasma. medRxiv. 2021:2021.01.26.21250224. https://doi.org/10.1038/s41586-021-03471-w PMID: 33780970.

16. Collier DA, Ferreira IATM, Kotagiri P, Dairir R, Lim E, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature. 2021. Epub 2021 Jun 30. https://doi.org/10.1038/s41586-021-03739-1 PMID: 34192737.

17. Edara VV, Norwood C, Floyd K, Lai L, Davis-Gardiner ME, Hudson WH, et al. Infection- and vaccine-induced antibody binding and neutralization of the B.1.1.7 SARS-CoV-2 variant. Cell Host Microbe. 2021; 29(4):516–27.e3. Epub 2021 Mar 20. https://doi.org/10.1016/j.chom.2021.03.009 PMID: 33798491; PubMed Central PMCID: PMC7980225.

18. Dejnirattisai W, Zhou D, Supasa P, Liu C, Mentzer AJ, Ginn HM, et al. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell. 2021; 184(11):2939–54.e9. Epub 2021 Mar 30. https://doi.org/10.1016/j.cell.2021.03.055 PMID: 33852911; PubMed Central PMCID: PMC8008340.

19. Abu-Raddad LJ, Chemaitelly H, Butt AA. Vaccination NSGfC -. Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. N Engl J Med. 2021. Epub May 5. https://doi.org/10.1056/NEJMc2104974 PubMed Central PMCID: PMC8117967. PMID: 33951357.
20. Shinde V, Bhikha S, Hoosain Z, Archary M, Bhorat Q, Fairlie L, et al. Efficacy of NVX-CoV2373 Covid-19 Vaccine against the B.1.351 Variant. N Engl J Med. 2021. Epub 2021 May 5. https://doi.org/10.1056/NEJMoa2103055 PMID: 33951374.

21. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, et al. Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant. N Engl J Med. 2021; 384(20):1885–98. Epub 2021 Mar 16. https://doi.org/10.1056/NEJMoa2102214 PMID: 33725432; PubMed Central PMCID: PMC7993410.

22. Amanat F, White KM, Miorin L, Strohmeier S, McMahon M, Meade P, et al. An In Vitro Microneutralization Assay for SARS-CoV-2 Serology and Drug Screening. Curr Protoc Microbiol. 2020; 58(1):e108. https://doi.org/10.1002/cpmt.108 PMID: 32583083.

23. Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD, et al. Mapping the antigenic and genetic evolution of influenza virus. Science. 2004; 305(5682):371–6. https://doi.org/10.1126/science.1079211 PMID: 15218094.

24. Sheikh A, McMenamin J, Taylor B, Robertson C, Collaborators PHSatEI. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. Lancet 2021; 397(10293):2461–2. Epub 2021 Jun 14. https://doi.org/10.1016/S0140-6736(21)01358-1 PMID: 34139198; PubMed Central PMCID: PMC8201647.

25. Bernai JL, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 variant. medRxiv. 2021.05.22.21257658. https://doi.org/10.1101/2021.05.22.21257656.

26. Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakison A, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell. 2021. Epub 2021 Jun 17. https://doi.org/10.1016/j.cell.2021.06.020 PMID: 34242578; PubMed Central PMCID: PMC8218332.

27. Carreño JM, Alshammary H, Singh G, Raskin A, Amanat F, Amoako A, et al. Evidence for retained spike-binding and neutralizing activity against emerging SARS-CoV-2 variants in serum of COVID-19 mRNA vaccine recipients. EBioMedicine. 2021; 73:103626. Epub 2021 Oct 20. https://doi.org/10.1016/j.ebiom.2021.103626 PMID: 34688034; PubMed Central PMCID: PMC8527879.

28. Earlie KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine. 2021. Epub 2021 May 24. https://doi.org/10.1016/j.vaccine.2021.05.063 PMID: 34210573.

29. Khoury DS, Cromer D, Reynolds A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021. Epub 2021 May 17. https://doi.org/10.1038/s41590-021-01377-8 PMID: 34002089.

30. Tarke A, Sidney J, Methot N, Zhang Y, Dan JM, Goodwin B, et al. Negligible impact of SARS-CoV-2 variants on CD4+ and CD8+ T cell reactivity in COVID-19 exposed donors and vaccinees. bioRxiv. 2021:2021.02.27.433180. https://doi.org/10.1126/science.1097211 PMID: 34002089; PubMed Central PMCID: PMC8700057.

31. Dilillo DJ, Tan GS, Palese P, Ravetch JV. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo. Nat Med. 2014; 20(2):143–51. https://doi.org/10.1038/nm.3443 PMID: 24412922.

32. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018; 19(11):1169–78. Epub 2018 Oct 17. https://doi.org/10.1038/s41590-018-0233-9 PMID: 30033617.

33. Saphire EO, Schendel SL, Fusco ML, Gangavarapu K, Gunn BM, Wec AZ, et al. Systematic Analysis of Monoclonal Antibodies against Ebola Virus GP Defines Features that Contribute to Protection. Cell. 2018; 174(4):938–52.e13. https://doi.org/10.1016/j.cell.2018.07.033 PMID: 30096313; PubMed Central PMCID: PMC6102396.

34. Asthagiri Anurakumar G, Ioanou A, Wohlbold TJ, Meade P, Aslam S, Amanat F, et al. Broadly Cross-Reactive, Nonneutralizing Antibodies against Influenza B Virus Hemagglutinin Demonstrate Effector Function-Dependent Protection against Lethal Viral Challenge in Mice. J Virol. 2019; 93(6). Epub 2019 May 22. https://doi.org/10.1128/JVI.01696-18 PMID: 30626682; PubMed Central PMCID: PMC6401450.

35. Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 serocconversion in humans. Nat Med. 2020; 26(7):1033–6. Epub 2020 May 14. https://doi.org/10.1038/s41591-020-0913-5 PMID: 32398876; PubMed Central PMCID: PMC8183627.

36. Margine I, Palese P, Krammer F. Expression of functional recombinant hemagglutinin and neuraminidase proteins from the novel H7N9 influenza virus using the baculovirus expression system. J Virol Exp. 2013;(81):e51112. Epub 2013 Dec 5. https://doi.org/10.3791/51112 PMID: 24300384; PubMed Central PMCID: PMC3970794.

37. Wohlbold TJ, Nachbagauer R, Xu H, Tan GS, Hirsh A, Brokstad KA, et al. Vaccination with adjuvanted recombinant neuraminidase induces broad heterologous, but not heterosubtypic, cross-protection.
against influenza virus infection in mice. mBio. 2015; 6(2):e02556. Epub 2015 Mar 12. https://doi.org/10.1128/mBio.02556-14 PMID: 25759506; PubMed Central PMCID: PMC4453582.

38. Harrington WE, Trakhimets O, Andrade DV, Dambrauskas N, Raappana A, Jiang Y, et al. Rapid decline of neutralizing antibodies is associated with decay of IgM in adults recovered from mild COVID-19. Cell Rep Med. 2021; 2(4):100253. Epub 2021 Apr 5. https://doi.org/10.1016/j.xcrm.2021.100253 PMID: 33842901; PubMed Central PMCID: PMC8020863.

39. Amanat F, Thapa M, Lei T, Ahmed SMS, Adelsberg DC, Carreno JM, et al. SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. Cell. 2021. Epub 2021 Jul 1. https://doi.org/10.1016/j.cell.2021.06.005 PMID: 33842901; PubMed Central PMCID: PMC8185186.

40. Rathnasighe R, Strohmeier S, Amanat F, Gillespie VL, Krammer F, Garcia-Sastre A, et al. Comparison of Transgenic and Adenovirus hACE2 Mouse Models for SARS-CoV-2 Infection. bioRxiv. 2020. Epub 2020 Jul 18. https://doi.org/10.1101/2020.07.06.190066 PMID: 32676603; PubMed Central PMCID: PMC7359525.

41. Amanat F, Duehr J, Huang C, Paessler S, Tan GS, Krammer F. Monoclonal Antibodies with Neutralizing Activity and Fc-Effecter Functions against the Machupo Virus Glycoprotein. J Virol. 2020; 94(5). Epub 2019 Dec 6. https://doi.org/10.1128/JVI.01741-19 PMID: 31801871; PubMed Central PMCID: PMC7022345.

42. Amanat F, Strohmeier S, Rathnasighe R, Schotsaert M, Coughlan L, Garcia-Sastre A, et al. Introduction of Two Prolines and Removal of the Polybasic Cleavage Site Lead to Higher Efficacy of a Recombinant Spike-Based SARS-CoV-2 Vaccine in the Mouse Model. mBio. 2021; 12(2). Epub 2021 Mar 4. https://doi.org/10.1128/mBio.02648-20 PMID: 33853892; PubMed Central PMCID: PMC8092267.

43. Sun W, Leist SR, McCroskery S, Liu Y, Slamanig S, Oliva J, et al. Newcastle disease virus (NDV) expressing the spike protein of SARS-CoV-2 as a live virus vaccine candidate. EBioMedicine. 2020; 62:103132. Epub 2020 Nov 25. https://doi.org/10.1016/j.ebiom.2020.103132 PMID: 33232870; PubMed Central PMCID: PMC7679520.