Check for updates

OPEN

mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant

Henning Gruell [1,7], Kanika Vanshylla [1,7], Pinkus Tober-Lau [2], David Hillus [2], Philipp Schommers[1,3], Clara Lehmann[3,4,5], Florian Kurth [2,6,7], Leif E. Sander [2,7] and Florian Klein [1,4,5,7] ✉

The Omicron variant of SARS-CoV-2 is causing a rapid increase in infections across the globe. This new variant of concern carries an unusually high number of mutations in key epitopes of neutralizing antibodies on the viral spike glycoprotein, suggesting potential immune evasion. Here we assessed serum neutralizing capacity in longitudinal cohorts of vaccinated and convalescent individuals, as well as monoclonal antibody activity against Omicron using pseudovirus neutralization assays. We report a near-complete lack of neutralizing activity against Omicron in polyclonal sera from individuals vaccinated with two doses of the BNT162b2 COVID-19 vaccine and from convalescent individuals, as well as resistance to different monoclonal antibodies in clinical use. However, mRNA booster immunizations in vaccinated and convalescent individuals resulted in a significant increase of serum neutralizing activity against Omicron. This study demonstrates that booster immunizations can critically improve the humoral immune response against the Omicron variant.

Most approved coronavirus disease 2019 (COVID-19) vaccines are based on transient expression of the viral spike (S) glycoprotein (derived from the Wu01 strain) to induce severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-directed immunity[1]. Mutations in antibody epitopes on the spike protein can result in increased viral resistance to neutralizing antibodies and have been associated with reduced vaccine effectiveness[2]. Moreover, they are able to strongly impair the activity of monoclonal antibodies used for the treatment and prevention of COVID-19 (ref. [3]). Emerging viral variants that escape the antibody response can therefore threaten the success of critical measures against SARS-CoV-2 infection.

Shortly after its identification in the Gauteng province in South Africa, the Omicron variant of SARS-CoV-2 (BA.1 sublineage of B.1.1.529) was designated as a variant of concern (VOC) by the World Health Organization (WHO). Genomic surveillance and surrogate parameters (for example S gene target failure in diagnostic polymerase chain reaction tests) document a sharp rise in Omicron infections across the globe[4]. Moreover, the observation of increasing incidence in populations with a high prevalence of SARS-CoV-2 immunity as well as reports of re-infection suggest that the Omicron variant possesses potent immune evasion properties[5]. Compared with previously described VOCs, the Omicron variant is notable for its high number of non-synonymous mutations relative to the ancestral Wu01 strain of SARS-CoV-2. The majority of these mutations are located in the viral spike glycoprotein and include critical epitopes for SARS-CoV-2-neutralizing antibodies in the N-terminal domain and the receptor-binding domain (RBD). Several of these mutations have already been associated with resistance to SARS-CoV-2- or vaccine-induced neutralizing antibodies[3,6,7]. Therefore, the spread of the Omicron variant can have important implications for current strategies to prevent and treat COVID-19, and may require urgent public health interventions to limit transmission and morbidity.

To determine the susceptibility of the Omicron variant to vaccine-induced serum activity, we analyzed samples obtained from 30 individuals with no evidence of prior infection[8]. Samples were collected 1 month (median, 4 weeks; range, 3–6 weeks; 'Early' time point) after completion of a two-dose course of the BNT162b2 vaccine (Fig. 1a). Study participants had a median age of 49 years (range, 27–78 years) and a nearly equal sex distribution (57% female participants, 43% male participants; Supplementary Table 1). Neutralizing activity was determined using an established lentivirus-based pseudovirus assay. Results obtained in pseudovirus assays typically correlate well with those obtained against authentic virus[9]. Sera were tested against pseudoviruses expressing the spike proteins of the Wu01 vaccine strain, or of the Alpha (B.1.1.7), Delta (B.1.617.2), Beta (B.1.351), or Omicron VOCs (Fig. 1a and Supplementary Table 2). All samples showed neutralizing activity against the Wu01 strain with a geometric mean 50% inhibitory serum dilution (GeoMean ID_{50}) of 546 (Fig. 1a). Serum neutralizing activity against the Alpha, Delta, and Beta variant was decreased to GeoMean ID_{50}s of 331, 172, and 40, respectively (samples that did not achieve 50% inhibition at the lowest tested dilution of 10 were imputed to an ID_{50} of 5). Notably, only nine out of the 30 vaccinated individuals (30%) had detectable serum neutralizing activity against Omicron, resulting in a GeoMean ID_{50} of 8 (Fig. 1a), which was significantly lower than against the Beta variant ($P < 0.0001$), one of the most immune evasive variants previously described[2].

To investigate the change in serum neutralizing activity against the Omicron variant over time and assess the impact of a booster

[1]Laboratory of Experimental Immunology, Institute of Virology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany. [2]Department of Infectious Diseases and Respiratory Medicine, Charité – Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany. [3]Department I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany. [4]German Center for Infection Research, Partner site Bonn-Cologne, Cologne, Germany. [5]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany. [6]Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine and Department of Medicine I, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [7]These authors contributed equally: Henning Gruell, Kanika Vanshylla, Florian Kurth, Leif E. Sander, Florian Klein. ✉e-mail: florian.klein@uk-koeln.de
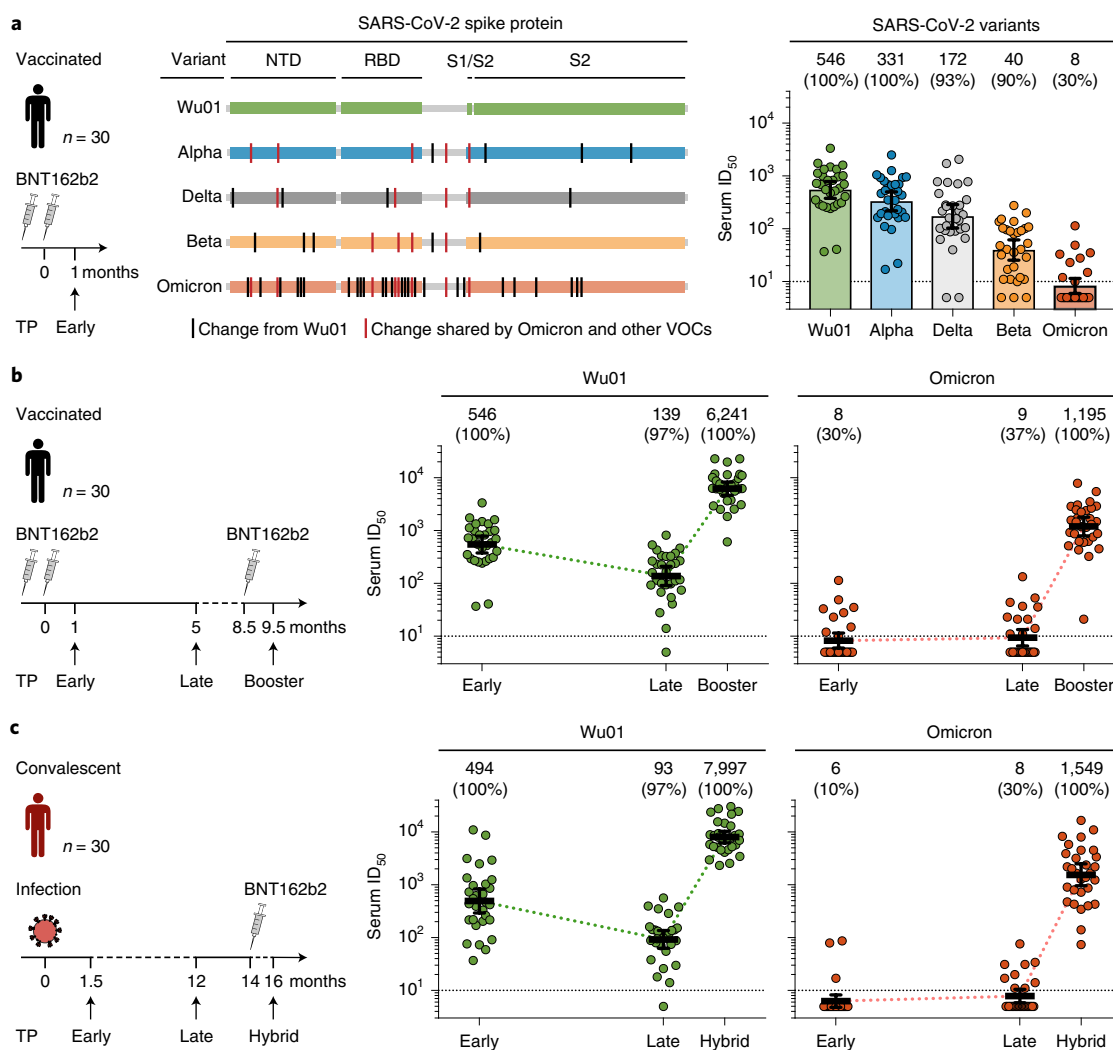
Fig. 1 | SARS-CoV-2-neutralizing serum activity in vaccinated and convalescent individuals. a, Neutralizing serum activity was determined in samples obtained 1 month after two doses of BNT162b2 against the ancestral Wu01 strain of SARS-CoV-2 and four VOCs. Residues with changes relative to the Wu01 strain are indicated by intersecting lines. Fifty percent inhibitory serum dilutions (ID_{50}s) were determined by pseudovirus neutralization assays. Bars indicate geometric mean ID_{50}s with 95% confidence intervals (CIs). Numbers above the graph indicate the geometric mean ID_{50}s, and the percentages of samples with detectable neutralizing activity above the lower limit of quantification (LLOQ) are given in parentheses. **b**, Serum ID_{50}s against the Wu01 strain and the Omicron variant of SARS-CoV-2 in a longitudinal cohort of 30 vaccinated individuals. Samples were collected at a median of 1 month (Early) and 5 months (Late) after two doses of BNT162b2, and 1 month after a subsequent single dose of BNT162b2 (Booster). Colored lines connect geometric mean ID_{50}s and error bars indicate the 95% CIs. **c**, Serum ID_{50}s against the Wu01 strain and the Omicron variant of SARS-CoV-2 in a longitudinal cohort of 30 COVID-19-convalescent individuals. Samples were collected at a median of 1.5 months (Early) and 12 months (Late) after diagnosis of SARS-CoV-2 infection, as well as 1.5 months after a single BNT162b2 vaccination (Hybrid). Colored lines connect geometric mean ID_{50}s and error bars indicate 95% CIs. In **a**, **b** and **c**, ID_{50}s below the LLOQ (ID_{50} of 10, indicated by the black dashed lines) were imputed to half the LLOQ ($ID_{50} = 5$). NTD, N-terminal domain; TP, time point.

vaccination, we analyzed longitudinal samples of the 30 vaccinees. Neutralizing serum activity against Wu01 and Omicron was determined at 5 months (median, 21 weeks; range, 19–31 weeks; 'Late' time point) after the second BNT162b2 vaccination, as well as at 1 month (median, 3 weeks; range, 2–12 weeks) after a single BNT162b2 booster dose (median, 41 weeks after the second vaccination; range, 30–44 weeks; 'Booster' time point; Fig. 1b). After completion of the two-dose course of the BNT162b2 vaccine, neutralizing activity against Wu01 decreased fourfold during the period of 5 months from a GeoMean ID_{50} of 546 to 139, but was strongly increased after booster vaccination (GeoMean ID_{50} of 6,241). Serum neutralizing activity against the Omicron variant after two vaccine doses remained low, with only 30–37% of the samples showing detectable neutralization, resulting in GeoMean ID_{50}s of 8 and 9

at the Early and Late time points, respectively. However, neutralizing serum activity to the Omicron variant increased by more than 100-fold after the booster dose of BNT162b2, resulting in a GeoMean ID_{50} of 1,195, and was detectable in all 30 participants (100%) (Fig. 1b). Notably, serum neutralizing activity against the Omicron variant following booster immunization was even higher than neutralizing titers against Wu01 after two doses of BNT162b2 (GeoMean ID_{50} of 1,195 versus 546; $P = 0.0003$).

We also analyzed the neutralizing serum response to the Omicron variant in a longitudinal cohort of 30 previously unvaccinated COVID-19 convalescent individuals (Supplementary Table 1)[10]. Study participants were followed for up to 16 months from the time of SARS-CoV-2 infection, which occurred between February and March 2020 (that is, prior to the emergence of

Fig. 2 | SARS-CoV-2-neutralizing activity of monoclonal antibodies. Fifty percent inhibitory concentrations (IC_{50}s) of monoclonal antibodies against the ancestral Wu01 strain of SARS-CoV-2 and four variants of concern determined by pseudovirus neutralization assays. $IC_{50} > 10\,\mu g\,ml^{-1}$ indicates failure to achieve 50% neutralizing activity at the highest tested antibody concentration of $10\,\mu g\,ml^{-1}$.

WHO-designated VOCs). Serum samples were collected at 1.5 months (range, 4–10 weeks; Early time point) and at 12 months (range, 49–59 weeks; Late time point) after the diagnosis of infection (Fig. 1c). In addition, serum was obtained 1 month (median, 6 weeks; range, 4–10 weeks) after a single dose of BNT162b2, resulting in a hybrid immunity acquired from a combination of infection and vaccination (median, 67 weeks; range, 62–71 weeks from disease onset; 'Hybrid' time point; Fig. 1c). Early after infection, neutralizing activity against Wu01 was variable, ranging in ID_{50}s from 37 to 11,008, with a GeoMean ID_{50} of 494 that decreased to 93 after 12 months (Late). Following a single BNT162b2 dose, all convalescent individuals had a strong increase in neutralizing serum activity resulting in a GeoMean ID_{50} of 7,997 against Wu01 (Hybrid). No neutralization (that is, no detectable ID_{50}) or only weak neutralizing activity was detected against the Omicron variant in samples collected from convalescent individuals at the Early and Late time points. However, a slight increase in neutralizing activity was observed at the Late time point in some individuals, potentially indicating ongoing affinity maturation resulting in antibodies capable of targeting a wider spectrum of SARS-CoV-2 variants (Supplementary Table 2). Despite the near-lack of neutralizing serum activity against the Omicron variant at a median of 12 months after SARS-CoV-2 infection (GeoMean ID_{50} of 8; Late time point), a single dose of BNT162b2 induced a strong increase, demonstrated by a GeoMean ID_{50} of 1,549 1 month after vaccination (Hybrid time point) (Fig. 1c). We conclude that the Omicron variant exerts substantial humoral immune escape in BNT162b2-vaccinated and convalescent individuals. However, high levels of neutralizing activity against the Omicron variant can be induced by a BNT162b2 booster immunization.

Finally, we investigated whether the high number of mutations in the Omicron variant affects the activity of SARS-CoV-2-neutralizing monoclonal antibodies, which have been demonstrated to effectively reduce COVID-19-associated morbidity and mortality[11,12]. To this end, we assessed the activity of nine monoclonal antibodies, encompassing antibodies authorized for clinical use (bamlanivimab, etesevimab, REGN10933 (casirivimab), REGN10987 (imdevimab) and S309 (sotrovimab)), an antibody in clinical investigation (DZIF-10c), and additional antibodies representative of different classes of antibodies targeting the RBD of the SARS-CoV-2 spike protein (P2B-2F6, C102 and Fab2-36) (Fig. 2). All antibodies were tested in parallel in the pseudovirus neutralization assay against the Wu01 strain as well as the Alpha, Delta, Beta, and Omicron variants.

Although all antibodies showed neutralizing activity against Wu01 and Alpha, only seven out of nine and five out of nine showed neutralizing activity against the Delta and Beta variants, respectively (Fig. 2). Notably, neutralizing activity against the Omicron variant was abolished in seven out of nine antibodies (Fig. 2). We conclude that the neutralizing activity of several monoclonal antibodies is markedly reduced against the Omicron variant and may limit treatment options for Omicron-induced COVID-19.

The rapid surge in Omicron variant infections poses a significant challenge to public health. This study demonstrates the marked resistance of the Omicron variant to serum neutralizing activity induced by two doses of the BNT162b2 vaccine or SARS-CoV-2 infection. Lower neutralizing titers have been associated with an increased risk of symptomatic COVID-19, suggesting that limited neutralizing activity against Omicron may result in increased risk of infection and higher burden of disease[13]. Whether cellular immunity will be effective in preventing severe disease after Omicron infection in the absence of a potent neutralizing antibody response remains to be determined[14,15]. Importantly, a single BNT162b2 booster immunization effectively induced a substantial increase in serum neutralization against the Omicron variant and resulted in neutralizing titers similar to those observed against Wu01 after two doses of BNT162b2. This analysis was limited to mostly healthy individuals receiving the BNT162b2 vaccine. However, the overall comparable immunogenicity of mRNA-1273 suggests that booster immunizations with both approved mRNA vaccines may yield similar results[16]. We speculate that the extent of the potent neutralizing response against the Omicron variant following booster vaccination may be a consequence of ongoing affinity maturation following initial vaccination or SARS-CoV-2 infection with the ancestral spike protein[17–19].

There is a critical need to make vaccines globally available to counter the emergence of novel variants. Although variant-specific vaccines and novel monoclonal antibodies may be required for optimal activity, the present data provide evidence that booster immunization with BNT162b2 can induce robust neutralization against the immune-evasive Omicron variant. However, higher levels of neutralizing serum activity might not necessarily prevent SARS-CoV-2 infection, as indicated by Omicron infections in boostered individuals. Finally, follow-up analyses will be required to determine the durability of the neutralizing antibody response to Omicron after booster immunization[20].

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/s41591-021-01676-0.

References

1. Krammer, F. SARS-CoV-2 vaccines in development. *Nature* **586**, 516–527 (2020).
2. Madhi, S. A. et al. Efficacy of the ChAdOx1 nCoV-19 COVID-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* **384**, 1885–1898 (2021).
3. Planas, D. et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature* **596**, 276–280 (2021).
4. Scott, L. et al. Track Omicron's spread with molecular data. *Science* **374**, 1454–1455 (2021).
5. Pulliam, J. R. C. et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. Preprint at https://doi.org/10.1101/2021.11.11.21266068 (2021).
6. Schmidt, F. et al. High genetic barrier to SARS-CoV-2 polyclonal neutralizing antibody escape. *Nature* **600**, 512–516 (2021).

7. Greaney, A. J. 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* **29**, 463–476 (2021).
8. Tober-Lau, P. et al. Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. *Lancet Respir. Med.* **9**, e104–e105 (2021).
9. Sholukh, A. M. et al. Evaluation of cell-based and surrogate SARS-CoV-2 neutralization assays. *J. Clin. Microbiol.* **59**, e0052721 (2021).
10. Vanshylla, K. et al. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. *Cell Host Microbe* **29**, 917–929 (2021).
11. O'Brien, M. P. et al. Subcutaneous REGEN-COV antibody combination to prevent Covid-19. *N. Engl. J. Med.* **385**, 1184–1195 (2021).
12. Gupta, A. et al. Early treatment for Covid-19 with SARS-CoV-2 neutralizing antibody sotrovimab. *N. Engl. J. Med.* **385**, 1941–1950 (2021).
13. Khoury, D. S. et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205–1211 (2021).
14. Sette, A. & Crotty, S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* **184**, 861–880 (2021).
15. Goel, R. R. et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science* **374**, abm0829 (2021).
16. Garcia-Beltran, W. F. et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* **184**, 2372–2383 (2021).
17. Turner, J. S. et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature* **596**, 109–113 (2021).
18. Muecksch, F. et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. *Immunity* **54**, 1853–1868 (2021).
19. Gaebler, C. et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639–644 (2021).
20. Goldberg, Y. et al. Waning immunity after the BNT162b2 vaccine in Israel. *N. Engl. J. Med.* **385**, e85 (2021).

Methods

Study design. Samples from COVID-19-convalescent individuals were obtained under protocols approved by the ethics committee of the Medical Faculty of the University of Cologne (16–054 and 20–1187). Individuals with a history of polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection (as reported by the participants to investigators, either verbally or by providing a positive test certificate) were enrolled at the University Hospital Cologne (Germany) between April and May 2020, ≤10 weeks after diagnosis and/or symptom onset, and followed longitudinally. Except for one participant who was hospitalized during acute SARS-CoV-2 infection, all individuals reported having mild SARS-CoV-2 infection-related symptoms. Although written proof of positive PCR testing was not formally required for study participation, the detectable SARS-CoV-2-neutralizing activity in all participants at baseline (prior to the availability of COVID-19 vaccines) provided independent confirmation of previous SARS-CoV-2 infection. Participants in the convalescent cohort were followed longitudinally to evaluate the long-term immune response to SARS-CoV-2, and booster immunizations (that is, vaccinations in the previously unvaccinated convalescent individuals) were performed as part of routine care. Samples for analysis in this study were identified based on information provided by the participants during regular follow-up visits with regard to whether and which COVID-19 vaccine (that is, booster immunization) they had received at what time.

Samples from non-infected vaccinated individuals were obtained under protocols (EICOV, COVIMMUNIZE and COVIM) approved by the ethics committee of Charité – Universitätsmedizin Berlin (EA4/245/20 and EA4/244/20; EICOV and COVIMMUNIZE), and by the Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute) and the ethics committee of the state of Berlin (COVIM). Participants in the observational vaccinated cohort studies were followed longitudinally to evaluate the immune response to approved SARS-CoV-2 vaccinations. Participation was offered to health-care workers vaccinated at the Charité – Universitätsmedizin (Berlin, Germany) as well as to elderly individuals (≥70 years) at a general practice (Berlin, Germany) irrespective of medical condition. Initial vaccinations as well as booster immunizations were independently performed as part of routine care outside of the cohort study. Samples for analysis in this study were selected post-hoc (that is, after booster immunizations were performed). All participants underwent nucleic acid amplification testing for SARS-CoV-2 at the time of sampling, and all samples were tested for the presence of anti-nucleocapsid antibodies using the SeraSpot Anti-SARS-CoV-2 IgG microarray-based immunoassay (Seramun Diagnostica). Samples from individuals with a history of SARS-CoV-2 infection (as reported by participants to investigators) or confirmed infection (positive nucleic acid amplification test or anti-nucleocapsid antibodies) were not included for analysis of the vaccinated cohort in this study.

All participants in the convalescent and vaccinated cohorts provided written informed consent. Selection of samples and participants for this study was based on receipt of the identical vaccine (BNT162b2), on the availability of largely comparable sampling time points (before and after immunizations), on a comparable and broad range of individuals of different age, and on a comparable and balanced sex distribution. Summarized details on the participants analyzed in this study are listed in Supplementary Table 1.

Cloning of SARS-CoV-2 spike constructs. The Omicron (BA.1 sublineage of B.1.1.529) spike construct was based on amino acid substitutions observed in initial isolates (EPI_ISL_6640916, EPI_ISL_6640917, EPI_ISL_6640919) and it carries the following changes in the spike protein compared with the Wu01 strain (EPI_ISL_406716): A67V, Δ69–70, T95I, G142D, Δ143–145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K and L981F. Codon-optimized overlapping gene fragments (Thermo Fisher) were assembled and cloned into the pCDNA3.1/V5-HisTOPO vector (Thermo Fisher, cat. no. K480001) using the NEBuilder HiFi DNA Assembly Kit (New England Biolabs, cat. no. E5520S). The Alpha (B.1.1.7, with Δ69–70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H changes), Beta (B.1.351, with D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G and A701V changes), and Delta (B.1.617.2, with T19R, G142D, Δ156–7, R158G, L452R, T478K, D614G, P681R and D950N changes) spike variants were produced by PCR-induced mutagenesis, and the assembly of PCR products was performed using the NEBuilder HiFi DNA Assembly Kit. All plasmid sequences were confirmed by sequencing.

Monoclonal antibodies. Antibodies S309 (ref. [21]), C102 (ref. [22]), Fab2-36 (ref. [23]) and P2B-2F6 (ref. [24]) were produced in 293-6E cells (National Research Council of Canada) by co-transfection of heavy and light chain expression plasmids using 25 kDa branched polyethylenimine (Sigma-Aldrich, cat. no. 408727). Following 6–7 days of incubation at 37 °C and 6% CO_2 under constant shaking at 110 r.p.m. in FreeStyle Expression Medium (Thermo Fisher, cat. no. 12338001) supplemented with 0.2% penicillin/streptomycin (Thermo Fisher, cat. 15140122), culture supernatants were collected, clarified by centrifugation and filtration (0.45 μm), and incubated with Protein G Sepharose 4 FastFlow (GE Life Sciences, cat. no. 17061805). Subsequently, antibodies were eluted from Protein G beads using

0.1 M glycine (pH 3.0) in chromatography columns (Bio-Rad) and buffered in 1 M Tris (pH 8.0). Final buffer exchange to PBS was performed using Amicon spin membranes (Millipore). Antibodies REGN10933 and REGN10987 (ref. [25]) were produced and kindly provided by Boehringer Ingelheim. Antibodies etesevimab (also known as CB6, ref. [26]), bamlanivimab (also known as LY-CoV555, ref. [27]), and DZIF-10c (derived from antibody HbnC3t1p1_F4, refs. [28,29]) were obtained from stocks of clinical product.

Pseudovirus neutralization assays. Pseudovirus neutralization assays were performed using a single-round infection lentivirus-based system[10,30]. Pseudovirus particles were generated in HEK293T cells by co-transfection of plasmids encoding for the SARS-CoV-2 spike protein, HIV-1 Tat, HIV-1 Gag/Pol, HIV-1 Rev, and luciferase followed by an internal ribosome entry site (IRES) and ZsGreen using FuGENE 6 Transfection Reagent (Promega, cat. no. E2691). Culture supernatants were changed to fresh medium 24 h after transfection, and pseudovirus-containing supernatants were collected at 48–72 h after transfection. Following centrifugation and filtration (0.45 μm), pseudoviruses were stored at −80 °C until use. Pseudoviruses were titrated by infecting 293T-ACE2 cells, and the luciferase activity was assessed, following a 48 h incubation period at 37 °C and 5% CO_2, by addition of luciferin/lysis buffer (10 mM MgCl₂ (cat. no. M8266), 0.3 mM ATP (cat. no. A26209), 0.5 mM Coenzyme A (cat. no. C3144), 17 mM IGEPAL (cat. no. I8896) (all Sigma-Aldrich), and 1 mM D-Luciferin (GoldBio, cat. no. LUCNA-1G) in Tris-HCL) using a microplate reader (Berthold). Pseudovirus dilutions resulting in an at least 1,000-fold difference in relative light units (RLUs) between infected and non-infected 293T-ACE2 cells were used for neutralization assays.

Serum samples were collected by centrifugation and stored at −80 °C until analysis. Serum samples were heat-inactivated at 56 °C for 45 min prior to use. Serial dilutions of serum (1:3 dilution series with a starting dilution of 1:10) and monoclonal antibodies (1:5 dilution series with a starting concentration of 10 μg ml⁻¹) were co-incubated with pseudovirus supernatants for 1 h at 37 °C prior to the addition of 293T-ACE2 cells. Following a 48 h incubation at 37 °C and 5% CO_2, luciferase activity was determined as described above. Serum samples were tested in single dilution series and monoclonal antibodies were tested in duplicates. Background RLUs of non-infected cells were subtracted, and the 50% inhibitory serum dilution (ID_{50}) and 50% inhibitory concentration of monoclonal antibodies were determined as the serum dilution and antibody concentration, respectively, resulting in a 50% RLU reduction compared with virus-infected untreated control cells. This was assessed using a non-linear fit model to plot an agonist versus normalized dose response curve with variable slope using the least squares fitting method in GraphPad Prism 7.0 (GraphPad).

Statistical methods. Comparison of neutralizing titers between different variants was performed using a two-tailed Wilcoxon matched-pairs signed rank test. Serum samples that did not achieve 50% inhibition at the lowest tested dilution of 10 (lower limit of quantification, LLOQ) were imputed to half of the LLOQ ($ID_{50} = 5$) for graphical representation and statistical evaluation.

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

Data availability

All data are included in the figures and supplementary information. Requests for additional data should be directed to the corresponding author and may be subject to restrictions based on data and privacy protection regulations and/or require a Material Transfer Agreement (MTA).

References

21. Pinto, D. et al. Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290–295 (2020).
22. Robbiani, D. F. et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437–442 (2020).
23. Wang, P. et al. A monoclonal antibody that neutralizes SARS-CoV-2 variants, SARS-CoV, and other sarbecoviruses. *Emerg. Microbes Infect.* **11**, 147–157 (2022).
24. Ju, B. et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**, 115–119 (2020).
25. Hansen, J. et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010–1014 (2020).
26. Shi, R. et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* **584**, 120–124 (2020).
27. Jones, B. E. et al. The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2 infection in nonhuman primates. *Sci. Transl. Med.* **13**, eabf1906 (2021).
28. Halwe, S. et al. Intranasal administration of a monoclonal neutralizing antibody protects mice against SARS-CoV-2 infection. *Viruses* **13**, 1498 (2021).

29. Kreer, C. et al. Longitudinal isolation of potent near-germline SARS-CoV-2-neutralizing antibodies from COVID-19 patients. *Cell* **182**, 843–854 (2020).
30. Crawford, K. H. D. et al. Protocol and reagents for pseudotyping lentiviral particles with SARS-CoV-2 spike protein for neutralization assays. *Viruses* **12**, 513 (2020).

Competing interests

H.G., K.V. and F. Klein are listed as inventors on pending patent application(s) on SARS-CoV-2-neutralizing antibodies filed by the University of Cologne. All other authors have no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01676-0.

Correspondence and requests for materials should be addressed to Florian Klein.

Peer review information *Nature Medicine* thanks Stephen Kent, Xiaoying Shen and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Alison Farrell was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Reprints and permissions information is available at www.nature.com/reprints.

nature portfolio

Corresponding author(s):   Florian Klein

Last updated by author(s):   Dec 21, 2021

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

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 was used.
Data analysis	Neutralization data was analyzed using GraphPad Prism version 7.0

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 Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are included in the figure, table, or supplement of this manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences   ☐ Behavioural & social sciences   ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No formal sample size calculation was performed. 30 participants from the convalescent and vaccinated cohorts were selected based on sample availability and similar distributions of age and gender.
Data exclusions	No data was excluded from analysis. Serum samples that did not achieve 50% inhibition at the lowest tested dilution of 10 (lower limit of quantification, LLOQ) were imputed to ½ of the LLOQ (ID50=5) for graphical representation and statistical evaluation.
Replication	Each serum sample was tested once against each analyzed pseudovirus strain in a single dilution series. Monoclonal antibodies were tested in technical duplicates against each pseudovirus strain and the average IC50 values are reported.
Randomization	Serum samples were obtained as part of longitudinal observational cohorts assessing the immune response after convalescence or after vaccination provided as routine care. Any vaccination was provided outside of the study protocols, so randomization was not applicable.
Blinding	Participants were enrolled into open-label observational cohorts. Vaccinations were not provided as part of the study protocols but as routine care. As a consequence, neither participants nor investigators were blinded to the receipt of vaccinations.

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

Antibodies

Antibodies used	Bamlanvimab (Jones et al.; PDB entry 7KMG) Etesevimab (Shi et al., PDB entry 7C01) REGN10933 (casirivimab) (Hanssen et al., PDB entry 6XDG) REGN10987 (imdevimab) (Hanssen et al., PDB entry 6XDG) S309 (sotrovimab) (Pinto et al., PDB entry 6WPS) C102 (Robbiani et al., PDB entry 7K8M) Fab2-36 (Liu et al., PDB entry 7N5H) DZIF-10c (Kreer et al.; Halwe et al.; GenBank IDs QKY76686.1 and QKY76714.1) P2B-2F6 (Bin Ju et al., PDB entry 7BWJ)
Validation	Plasmids for antibody production were verified by sequencing. For Bamlanvimab, Etesevimab, and DZIF-10c, batches produced for clinical application were used.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells (ATCC Catalog# CRL-11268) 293T-ACE2 cells (BEI resources Catalog # 52511)

293-6E cells (NRC file 11565)

Authentication	Cell lines were not authenticated.
Mycoplasma contamination	Cell lines were not checked for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	COVID-19 convalescent cohort: 30 convalescent individuals, 14 males and 16 females, with a median age of 52 years. Individuals were SARS-CoV-2-infected in February or March 2020. 29/30 convalescent individuals received one dose of BNT162b2 at a median of 15 months after disease onset. One individual received an additional dose of BNT162b2 at 3 weeks after the first dose. Vaccinated cohort: 30 vaccinated individuals, 13 males and 17 females, with a median age of 49 years. All individuals received 2 doses of BNT162b2, approximately three weeks apart followed by a third dose at a median of 8.5 months after the second dose.
Recruitment	COVID-19 convalescent cohort: Participants were invited to participate in the cohort when fulfilling the inclusion criteria of i.) age ≥18 years, ii.) history of SARS-CoV-2 positive polymerase chain reaction (PCR) from a respiratory swab or collected sputum, and/or iii.) an onset of COVID-19 specific symptoms more than 3 weeks ago. Participants enrolled in this cohort were followed longitudinally in an outpatient study clinic to assess the longitudinal changes in the immune response to SARS-CoV-2 after vaccination. Participants received financial compensation and were informed about their antibody levels to SARS-CoV-2, when assessed. It is possible that individuals with long-term symptoms would show higher retention rates, but this would not affect immunogencity results and the conclusions drawn. Vaccinated cohort: Samples were obtained from two longitudinal cohorts of vaccinated individuals that analyze the long-term immune response to COVID-19 vaccines. The cohorts were composed of vaccinated health care workers that were offered participation, as well as of elderly individuals (70 years and higher) of a general practice that were offered participation irrespective of medical conditions. Participants underwent NAAT at times of sampling. No samples from individuals with positive SARS-CoV-2 testing by NAAT or anti-nucleocapsid serology or history of SARS-CoV-2 infection were considered for analysis in this study. Participants received financial compensation for their participation. All participants in all cohorts provided written informed consent for their participation. Neither initial vaccinations nor booster immunizations were offered within the observational studies, and all vaccinations were performed within routine care. Samples and participants for analysis were selected post-hoc (i.e., after booster immunizations had been performed within routine care) and chosen to be overall comparable (administered vaccines, temporal relationship of sampling visits to vaccinations, sex and age distribution). Overall, participants were relatively healthy which may be associated with a higher response to vaccinations compared to less healthy cohorts.
Ethics oversight	COVID-19-convalescent samples were obtained under protocols approved by the ethics committee (EC) of the Medical Faculty of the University of Cologne (16-054 and 20-1187). Samples from non-infected vaccinated individuals were obtained under protocols (EICOV, COVIMMUNIZE, and COVIM) approved by the EC of Charité - Universitätsmedizin Berlin (EA4/245/20 and EA4/244/20; EICOV and COVIMMUNIZE), and by the Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute) and the EC of the state of Berlin (COVIM).

Note that full information on the approval of the study protocol must also be provided in the manuscript.