Check for updates

Neutralization of SARS-CoV-2 Variants of Concern Harboring Q677H

Cong Zeng,[a,b] John P. Evans,[a,b,c] Julia N. Faraone,[a,b,c] Panke Qu,[a,b] Yi-Min Zheng,[a,b] (ID) Linda Saif,[d,h] Eugene M. Oltz,[e] Gerard Lozanski,[f] Richard J. Gumina,[g] Shan-Lu Liu[a,b,e,h]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, Ohio, USA
[b]Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio, USA
[c]Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, Ohio, USA
[d]Center for Food Animal Health, Animal Sciences Department, OARDC, College of Food, Agricultural and Environmental Sciences and Veterinary Preventive Medicine Department, College of Veterinary Medicine, The Ohio State University, Wooster, Ohio, USA
[e]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, Ohio, USA
[f]Department of Pathology, The Ohio State University, Columbus, Ohio, USA
[g]Division of Cardiovascular Medicine, Department of Internal Medicine, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, USA
[h]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, Ohio, USA

Cong Zeng and John P. Evans contributed equally to this work. Order was determined based on seniority.

ABSTRACT The sensitivity of SARS-CoV-2 variants of concern (VOCs) to neutralizing antibodies has largely been studied in the context of key receptor binding domain (RBD) mutations, including E484K and N501Y. Little is known about the epistatic effects of combined SARS-CoV-2 spike mutations. We now investigate the neutralization sensitivity of variants containing the non-RBD mutation Q677H, including B.1.525 (Nigerian isolate) and Bluebird (U.S. isolate) variants. The effect on neutralization of Q677H was determined in the context of the RBD mutations and in the background of major VOCs, including B.1.1.7 (United Kingdom, Alpha), B.1.351 (South Africa, Beta), and P1-501Y-V3 (Brazil, Gamma). We demonstrate that the Q677H mutation increases viral infectivity and syncytium formation, as well as enhancing resistance to neutralization for VOCs, including B.1.1.7 and P1-501Y-V3. Our work highlights the importance of epistatic interactions between SARS-CoV-2 spike mutations and the continued need to monitor Q677H-bearing VOCs.

IMPORTANCE SARS-CoV-2, the causative agent of COVID-19, is rapidly evolving to be more transmissible and to evade acquired immunity. To date, most investigations of SARS-CoV-2 variants have focused on RBD mutations. However, the impact of non-RBD mutations and their synergy with studied RBD mutations are poorly understood. Here, we examine the role of the non-RBD Q677H mutation arising in many SARS-CoV-2 lineages, including VOCs. We demonstrate that the Q677H mutation enhances viral infectivity and confers neutralizing antibody resistance, particularly in the background of other SARS-CoV-2 VOCs.

KEYWORDS Q677H, SARS-CoV-2, spike, neutralization, variant of concern

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve into new variants of concern (VOCs) with increased transmissibility, pathogenesis, and vaccine resistance (1). Such mutations can have drastic effects on viral spread, as illustrated by the D614G mutation, which emerged early in the pandemic and is now present in nearly all circulating SARS-CoV-2 strains (2, 3). As global vaccination efforts are under way, monitoring the immune escape of VOCs remains a critical priority. Neutralizing antibodies, or antibodies that directly block virus entry (4), are a key

Address correspondence to Shan-Lu Liu, liu.6244@osu.edu.

measure of protection against SARS-CoV-2 (5–7). Given that neutralizing antibodies often target the receptor binding domain (RBD) (8), recent studies on neutralizing antibody escape by VOCs, including the rapidly spreading B.1.1.7 (United Kingdom, Alpha), B.1.351 (South Africa, Beta), B.1.429 (United States), and P1 (P1-501Y-V3, Brazil, Gamma) variants (9–13), have focused on mutations in the RBD, including E484K, N501Y, and L452R, which have been shown to decrease the efficacy of mRNA vaccines to VOCs (14, 15).

In comparison, emerging B.1.525 (Nigeria) and Bluebird (United States) variants containing Q677H have received less attention, despite maintaining a strong prevalence in some parts of the United States, especially the Midwest and Southeast areas, as well as Nigeria (16–18). Both the B.1.525 and Bluebird variants harbor a key S1 non-RBD mutation, Q677H, with the B.1.525 variant also possessing the E484K RBD mutation (Fig. 1a). Importantly, the Q677H mutation is also present in some isolates of VOCs, including B.1.1.7, B.1.351, and P1 (19). The possible role of Q677H in modulating viral infectivity and SARS-CoV-2 sensitivity to antibody neutralization is currently unknown, a particular concern if it emerges in an existing VOC. Here, we examine the infectivity and neutralization of non-RBD Q677H-bearing variants and define its synergistic effects in the context of key RBD mutations.

Q677H enhances resistance to neutralizing antibodies. To examine neutralizing activity against SARS-CoV-2 variants, we utilized our previously reported intron-*Gaussia* luciferase-bearing lentiviral pseudotype-based neutralization assay (20) (see Text S1 in the supplemental material). We first determined the neutralizing activity of sera from 9 intensive care unit (ICU) COVID-19 patients and 9 hospitalized non-ICU patients against SARS-CoV-2 USA-WA1/2020 (wild type [WT]), D614G, and a panel of variants harboring the Q677H mutation (Fig. 1a to c). Sera were collected from both groups at least 14 days after symptom onset. For all neutralization assays, pseudotyped viruses were adjusted to comparable infectivity prior to neutralization—to prevent variations in infectivity from affecting virus neutralization. Notably, the single D614G mutant showed an increase in 50% neutralization titer (NT_{50}) compared to WT (Fig. 1b and c), likely because the D614G mutation stabilizes the "open" (RBD-exposed) spike conformation (21). The Bluebird and B.1.525 variants exhibited ~2.2-fold ($P < 0.01$)- and ~3.6-fold ($P < 0.01$)-reduced NT_{50}, respectively, compared with D614G for ICU patient samples (Fig. 1b), while non-ICU samples showed ~2.6-fold ($P < 0.01$)-reduced NT_{50} for B.1.525 with only ~10% reduction for Bluebird (Fig. 1c), likely due to the ~8.7-fold-lower titer of the non-ICU samples or variations in disease state at time of serum collection.

In examining the infectivity of the lentiviral pseudotypes (see Text S1 in the supplemental material), all D614G-containing variants showed enhanced infectivity, whereas E484K exhibited decreased infectivity (29%, $P < 0.01$) and B.1.525 showed an increase (41%, $P < 0.01$) compared to D614G (Fig. 1d). Interestingly, although the Q677H mutation exhibited no drastic effect on SARS-CoV-2 S cleavage (Fig. S1a and b), it appeared to increase the spike-induced syncytium formation (Fig. S1c and d), neutralization resistance (Fig. S1e), and infectivity (Fig. S1f).

We next tested the neutralization capacities of sera from 40 age-matched recipients of the Moderna mRNA-1273 ($n = 20$; mean age, 35.4 years) and Pfizer BNT162b2 ($n = 20$; mean age, 35.3 years) vaccines collected 3 to 4 weeks post-second dose (see Text S1 in the supplemental material). Again, all variants, including those containing Q677H, showed decreased neutralization compared with D614G. In particular, B.1.525 and E484K/Q677H/D614G exhibited 36.5% ($P < 0.001$) and 46.5% ($P < 0.001$), respectively, decreased NT_{50} relative to D614G (Fig. 1e). Of note, E484K, N501Y, and Q677H mutants conferred 38.3% ($P < 0.001$), 17.8%, and 11.1% decreased NT_{50}, respectively, compared with D614G (Fig. 1e and f).

Q677H enhances infectivity and neutralizing antibody resistance of prevalent VOCs. To examine the impact of the Q677H mutation emerging in major VOCs, we examined its possible role in the backgrounds of the B.1.1.7, B.1.351, and P1 variants. We found that introduction of the Q677H mutation to the B.1.1.7 and P1 spike
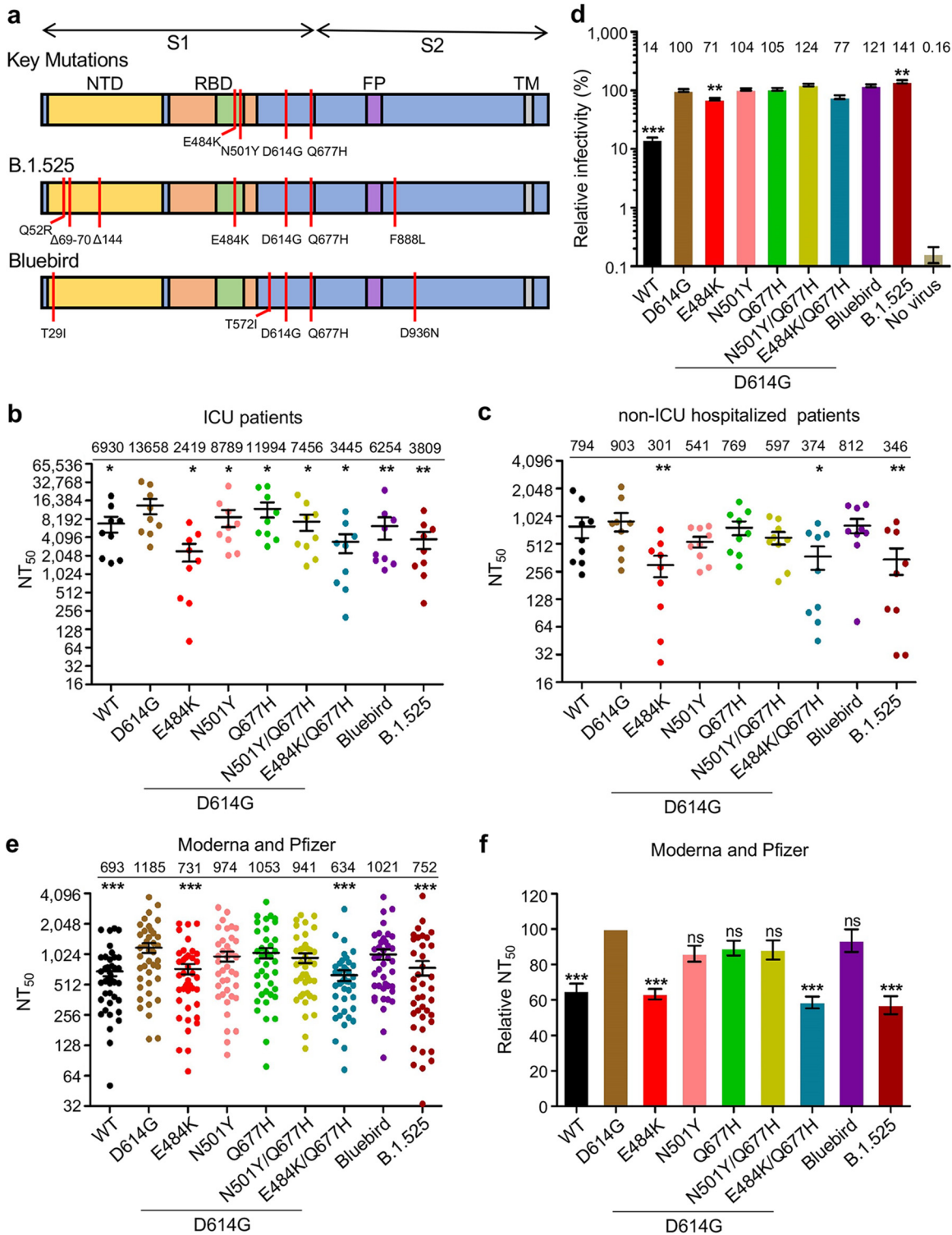
FIG 1 Neutralization of Q677H-bearing SARS-CoV-2 spike-pseudotyped lentivirus by convalescent-phase and vaccinee sera. (a) A schematic representation of the SARS-CoV-2 spike protein is presented that outlines the N-terminal domain (NTD), the receptor binding domain (RBD) with the receptor binding motif in green, the fusion peptide (FP), and transmembrane (TM) regions. Key mutations E484K, N501Y, D614G, and Q677H are indicated. Spike mutations for the B.1.525 and Bluebird variants are shown. (b and c) Luciferase readout from HEK293T-ACE2 cells infected with pseudotyped HIV-1-NL4-3-inGluc virus that had been incubated with serial dilutions of patient serum was used to determine NT_{50} values (also indicated on the top) for sera from 9 COVID-19 ICU patients and 9 non-ICU hospitalized COVID-19 patients against the indicated SARS-CoV-2 spike pseudotyped viruses, including B.1.525 and Bluebird. (d) Infectivity of the lentiviral pseudotypes bearing the indicated spikes of interest on HEK293T-ACE2 cells. Relative infectivity (also indicated on the top) was calculated by setting the value of D614G to 100. (e and f) NT_{50} values and NT_{50} values relative to the D614G

proteins further reduced the NT_{50} of sera from mRNA vaccinees (8 Moderna and 8 Pfizer) by ~21.9% ($P < 0.05$) and ~29.0% ($P < 0.001$), respectively (Fig. 2a). Additionally, the Q677H mutation increased the pseudotyping viral infectivity of B.1.1.7 by 2.5-fold ($P < 0.001$) and of P1 by 26.3% ($P < 0.001$) (Fig. 2b). Interestingly, no effect of Q677H on infectivity or neutralizing antibody resistance was observed for B.1.351 (Fig. 2a and b).

Given the impact and epistasis of the non-RBD mutation Q677H on infectivity and neutralization escape, we hypothesized that it may induce conformational changes in the spike protein. To test this, we performed virus neutralization in the presence of an RBD-binding monoclonal antibody, 2B04 (22), which is known to bind the receptor binding motif of the RBD and serves as a conformation-dependent antibody. 2B04 exhibited an ~50% reduction in neutralization of Q677H relative to WT and of Q677H/D614G relative to D614G (Fig. 2c), potentially indicating an alteration to RBD conformation. Unsurprisingly, spike proteins bearing the E484K mutation in the epitope of 2B04 (22) were not neutralized by 2B04 (Fig. 2c).

Moderna versus Pfizer neutralization of Q677H-containing variants. We compared the effects of age-matched Moderna and Pfizer mRNA vaccines on neutralization of all variants used in this study, including VOCs (Fig. 2d and e). Overall, the Moderna vaccine induced an ~52.7% higher NT_{50} than did the Pfizer vaccine (Fig. 2d; $P < 0.001$). In fact, the Moderna vaccine outperformed the Pfizer vaccine against each of the viruses tested in this study, in particular B.1.525, B.1.351, and P1 ($P < 0.01$) (Fig. 2e).

Discussion. Although the non-RBD mutation Q677H alone in SARS-CoV-2 spike led to only modest neutralization resistance, it increased viral infectivity and syncytium formation and, importantly, had an epistatic effect when paired with certain emerging RBD mutations present in VOCs. Q677 is situated in a disordered region near the critical RRAR furin-cleavage site of SARS-CoV-2 spike (16). Thus, it is possible that Q677H might alter protease processing or spike conformation, as suggested by the increased syncytium formation and reduced neutralization by 2B04 of the D614G/Q677H spike compared to D614G; however, no dramatic effect of Q677H on furin cleavage was observed. It is possible that the effect of Q677H is masked by the presence of other mutations, including D614G. Future structural studies are required to determine the exact mechanisms by which Q677H impacts infectivity and spike conformation.

In this study, we found that the E484K mutation had a greater impact on neutralization by convalescent-phase (~3.8-fold decrease) compared with vaccinee (~2-fold decrease) sera, consistent with recent reports (14). Moreover, we found that Q677H increased the infectivity and neutralizing antibody resistance of B.1.1.7 and P1 spike. These conclusions are further strengthened by the neutralization profiles of K484E and H677Q reversion mutants made in the backbone of B.1.525 and Bluebird variants, where a modest effect was observed for Q677H mutants compared to K484E (Fig. S2). These findings are critical as SARS-CoV-2 VOCs continue to evolve to increase their transmissibility and resistance to vaccinee sera, including the Delta variants (2, 23–25). Interestingly, we did not find increased infectivity and neutralizing antibody resistance for Q677H in the context of the B.1.351 variant, which could be due to its preexisting strong resistance to neutralization (23) or due to the presence of other compensatory mutations. Overall, our findings underscore the need to better understand epistatic interactions between RBD and non-RBD mutations in the spike as SARS-CoV-2 evolves in the face of new immunologic challenges.

FIG 1 Legend (Continued)
virus were determined for 40 vaccinee serum samples collected 3 to 4 weeks after their second dose of Moderna ($n = 20$) or Pfizer ($n = 20$) vaccine with significance being determined by one-way repeated-measures analysis of variance (ANOVA) with Bonferroni posttest. All comparisons were made against D614G; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.
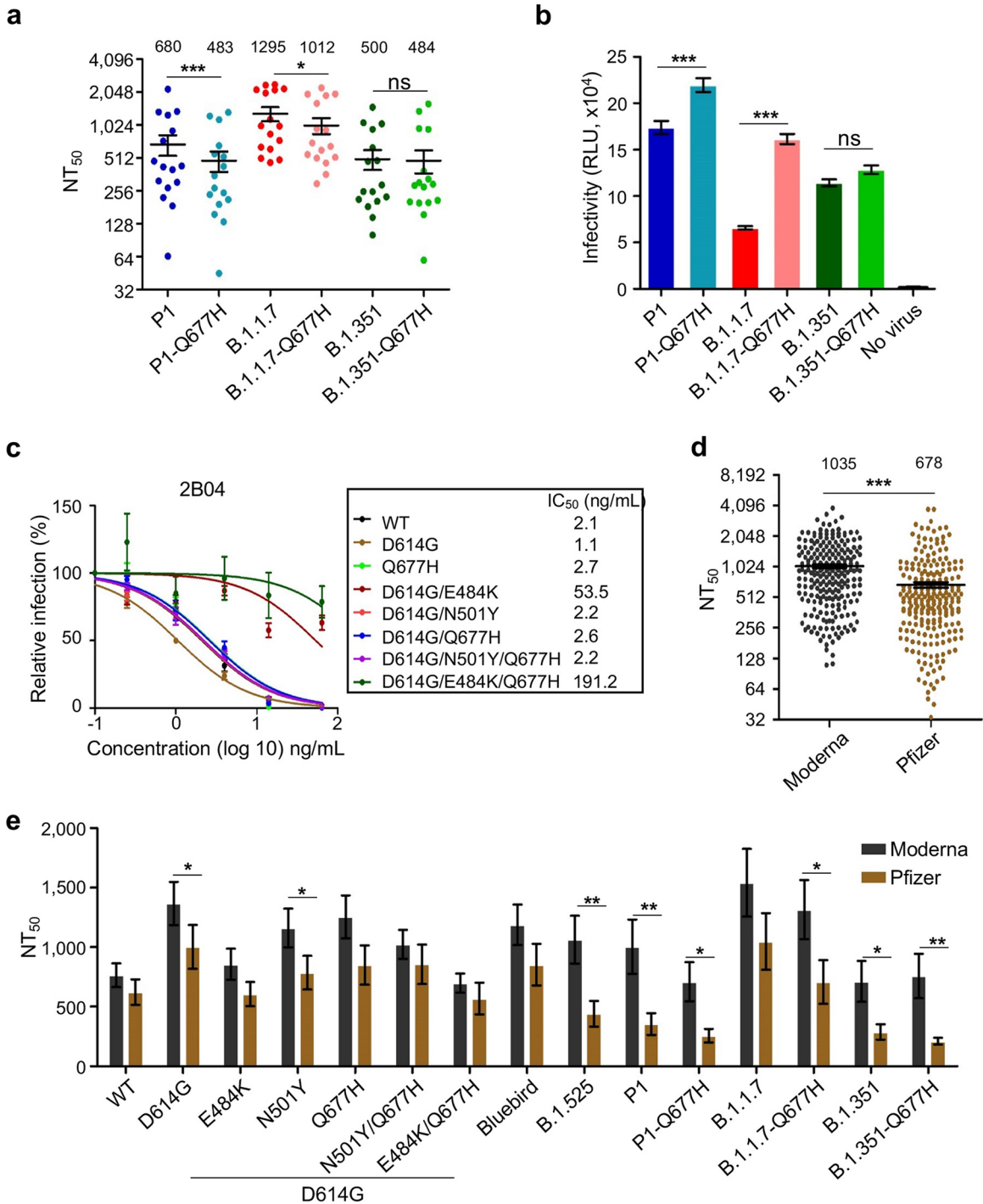
FIG 2 Comparison of the neutralization of VOCs B.1.1.7, B.1.351, and P1 with their counterparts containing Q677H, as well as comparison of the Moderna and Pfizer neutralizing antibody responses against Q677H-bearing variants. (a) NT_{50} values (indicated on the top) for mRNA vaccinee serum from 8 Moderna and 8 Pfizer samples, determined as in Fig. 1, against P1-501Y-V3 (Brazil), B.1.1.7 (United Kingdom), and B.1.351 (South Africa) variants with or without the introduction of the Q677H mutation. (b) Infectivity of indicated VOCs with or without Q677H. (c) Neutralization curves for monoclonal 2B04 are displayed with half-maximal inhibitory concentration (IC_{50}) values, with error bars representing standard error. (d and e) The NT_{50} values for Moderna and Pfizer samples were compared against all variants tested with statistical significance being determined by unpaired, one-tailed t test assuming equal variance (d) and for each variant tested with statistical significance being determined by multiple unpaired t test (e). Bars represent means with standard error, and mean NT_{50} are displayed at the top of the plots; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TEXT S1, DOCX file, 0.03 MB.
FIG S1, PDF file, 1.1 MB.
FIG S2, PDF file, 0.1 MB.

REFERENCES

1. Walensky RP, Walke HT, Fauci AS. 2021. SARS-CoV-2 variants of concern in the United States—challenges and opportunities. JAMA 325:1037–1038. https://doi.org/10.1001/jama.2021.2294.

2. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM, Freeman TM, de Silva TI, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, LaBranche CC, Saphire EO, Montefiori DC, Sheffield COVID-19 Genomics Group. 2020. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 182:812–827.e19. https://doi.org/10.1016/j.cell.2020.06.043.

3. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2021. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 592:116–121. https://doi.org/10.1038/s41586-020-2895-3.

4. Iwasaki A, Yang Y. 2020. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 20:339–341. https://doi.org/10.1038/s41577-020-0321-6.

5. Addetia A, Crawford KH, Dingens A, Zhu H, Roychoudhury P, Huang M-L, Jerome KR, Bloom JD, Greninger AL. 2020. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol 58:e02107-20. https://doi.org/10.1128/JCM.02107-20.

6. Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He W-T, Limbo O, Smith C, Song G, Woehl J, Yang L, Abbott RK, Callaghan S, Garcia E, Hurtado J, Parren M, Peng L, Ramirez S, Ricketts J, Ricciardi MJ, Rawlings SA, Wu NC, Yuan M, Smith DM, Nemazee D, Teijaro JR, Voss JE, Wilson IA, Andrabi R, Briney B, Landais E, Sok D, Jardine JG, Burton DR. 2020. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science 369:956–963. https://doi.org/10.1126/science.abc7520.

7. Hassan AO, Case JB, Winkler ES, Thackray LB, Kafai NM, Bailey AL, McCune BT, Fox JM, Chen RE, Alsoussi WB, Turner JS, Schmitz AJ, Lei T, Shrihari S, Keeler SP, Fremont DH, Greco S, McCray PB, Perlman S, Holtzman MJ, Ellebedy AH, Diamond MS. 2020. A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. Cell 182:744–753.e4. https://doi.org/10.1016/j.cell.2020.06.011.

8. Dejnirattisai W, Zhou D, Ginn HM, Duyvesteyn HME, Supasa P, Case JB, Zhao Y, Walter TS, Mentzer AJ, Liu C, Wang B, Paesen GC, Slon-Campos J, López-Camacho C, Kafai NM, Bailey AL, Chen RE, Ying B, Thompson C, Bolton J, Fyfe A, Gupta S, Tan TK, Gilbert-Jaramillo J, James W, Knight M, Carroll MW, Skelly D, Dold C, Peng Y, Levin R, Dong T, Pollard AJ, Knight JC, Klenerman P, Temperton N, Hall DR, Williams MA, Paterson NG, Bertram FKR, Siebert CA, Clare DK, Howe A, Radecke J, Song Y, Townsend AR, Huang K-YA, Fry EE, Mongkolsapaya J, Diamond MS, Ren J, Stuart DI, Screaton GR. 2021. The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell 184:2183–2200.e22. https://doi.org/10.1016/j.cell.2021.02.032.

9. Liu Y, Liu J, Xia H, Zhang X, Fontes-Garfias CR, Swanson KA, Cai H, Sarkar R, Chen W, Cutler M, Cooper D, Weaver SC, Muik A, Sahin U, Jansen KU, Xie X, Dormitzer PR, Shi P-Y. 2021. Neutralizing activity of BNT162b2-elicited serum. N Engl J Med 384:1466–1468. https://doi.org/10.1056/NEJMc2102017.

10. Shen X, Tang H, Pajon R, Smith G, Glenn GM, Shi W, Korber B, Montefiori DC. 2021. Neutralization of SARS-CoV-2 variants B.1.429 and B.1.351. N Engl J Med 384:2352–2354. https://doi.org/10.1056/NEJMc2103740.

11. Muik A, Wallisch A-K, Sänger B, Swanson KA, Mühl J, Chen W, Cai H, Maurus D, Sarkar R, Türeci Ö, Dormitzer PR, Şahin U. 2021. Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine–elicited human sera. Science 371:1152–1153. https://doi.org/10.1126/science.abg6105.

12. Planas D, Bruel T, Grzelak L, Guivel-Benhassine F, Staropoli I, Porrot F, Planchais C, Buchrieser J, Rajah MM, Bishop E, Albert M, Donati F, Prot M, Behillil S, Enouf V, Maquart M, Smati-Lafarge M, Varon E, Schortgen F, Yahyaoui L, Gonzalez M, De Sèze J, Péré H, Veyer D, Sève A, Simon-Lorière E, Fafi-Kremer S, Stefic K, Mouquet H, Hocqueloux L, van der Werf S, Prazuck T, Schwartz O. 2021. Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. Nat Med 27:917–924. https://doi.org/10.1038/s41591-021-01318-5.

13. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD, Graham BS, Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng Z, Huang Y, Ho DD. 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 593:130–135. https://doi.org/10.1038/s41586-021-03398-2.

14. Greaney AJ, Loes AN, Gentles LE, Crawford KH, Starr TN, Malone KD, Chu HY, Bloom JD. 2021. The SARS-CoV-2 mRNA-1273 vaccine elicits more RBD-focused neutralization, but with broader antibody binding within the RBD. bioRxiv https://doi.org/10.1101/2021.04.14.439844.

15. Chen RE, Zhang X, Case JB, Winkler ES, Liu Y, VanBlargan LA, Liu J, Errico JM, Xie X, Suryadevara N, Gilchuk P, Zost SJ, Tahan S, Droit L, Turner JS, Kim W, Schmitz AJ, Thapa M, Wang D, Boon ACM, Presti RM, O'Halloran JA, Kim AHJ, Deepak P, Pinto D, Fremont DH, Crowe JE, Corti D, Virgin HW, Ellebedy AH, Shi P-Y, Diamond MS. 2021. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nat Med 27: 717–726. https://doi.org/10.1038/s41591-021-01294-w.

16. Hodcroft EB, Domman DB, Snyder DJ, Oguntuyo KY, Van Diest M, Densmore KH, Schwalm KC, Femling J, Carroll JL, Scott RS, Whyte MM, Edwards MW, Hull NC, Kevil CG, Vanchiere JA, Lee B, Dinwiddie DL, Cooper VS, Kamil JP. 2021. Emergence in late 2020 of multiple lineages of SARS-CoV-2 Spike protein variants affecting amino acid position 677. medRxiv https://doi.org/10.1101/2021.02.12.21251658.

17. Ozer EA, Simons LM, Adewumi OM, Fowotade AA, Omoruyi EC, Adeniji JA, Dean TJ, Taiwo BO, Hultquist JF, Lorenzo-Redondo R. 2021. High prevalence of SARS-CoV-2 B.1.1.7 (UK variant) and the novel B.1.525 lineage in Oyo State, Nigeria. medRxiv https://www.medrxiv.org/content/medrxiv/early/2021/04/23/2021.04.09.21255206.full.pdf.

18. Tu H, Avenarius MR, Kubatko L, Hunt M, Pan X, Ru P, Garee J, Thomas K, Mohler P, Pancholi P. 2021. Distinct patterns of emergence of SARS-CoV-2 spike variants including N501Y in clinical samples in Columbus Ohio. bioRxiv https://www.biorxiv.org/content/10.1101/2021.01.12.426407v1.

19. Nagy A, Basiouni S, Parvin R, Hafez HM, Shehata AA. 2021. Evolutionary insights into the furin cleavage sites of SARS-CoV-2 variants from humans and animals. Arch Virol 166:2541–2549. https://doi.org/10.1007/s00705-021-05166-z.

20. Zeng C, Evans JP, Pearson R, Qu P, Zheng Y-M, Robinson RT, Hall-Stoodley L, Yount J, Pannu S, Mallampalli RK, Saif L, Oltz E, Lozanski G, Liu S-L. 2020. Neutralizing antibody against SARS-CoV-2 spike in COVID-19 patients, health care workers, and convalescent plasma donors. JCI Insight 5: e143213. https://doi.org/10.1172/jci.insight.143213.

21. Mansbach RA, Chakraborty S, Nguyen K, Montefiori DC, Korber B, Gnanakaran S. 2021. The SARS-CoV-2 spike variant D614G favors an open conformational state. Sci Adv 7:eabf3671. https://doi.org/10.1126/sciadv.abf3671.

22. Liu Z, VanBlargan LA, Bloyet L-M, Rothlauf PW, Chen RE, Stumpf S, Zhao H, Errico JM, Theel ES, Liebeskind MJ, Alford B, Buchser WJ, Ellebedy AH, Fremont DH, Diamond MS, Whelan SPJ. 2021. Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe 29:477–488.e4. https://doi.org/10.1016/j.chom.2021.01.014.

23. Zhou D, Dejnirattisai W, Supasa P, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn HME, Tuekprakhon A, Nutalai R, Wang B, Paesen GC, Lopez-Camacho C, Slon-Campos J, Hallis B, Coombes N, Bewley K, Charlton S, Walter TS, Skelly D, Lumley SF, Dold C, Levin R, Dong T, Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M, Belij-Rammerstorfer S, Gilbert S, James W, Carroll MW, Klenerman P, Barnes E, Dunachie SJ, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. 2021. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 184:2348–2361.e6. https://doi.org/10.1016/j.cell.2021.02.037.

24. Choe H, Farzan M. 2021. How SARS-CoV-2 first adapted in humans. Science 372:466–467. https://doi.org/10.1126/science.abi4711.

25. Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A, Nutalai R, Zhou D, Mentzer AJ, Zhao Y, Duyvesteyn HME, López-Camacho C, Slon-Campos J, Walter TS, Skelly D, Johnson SA, Ritter TG, Mason C, Costa Clemens SA, Gomes Naveca F, Nascimento V, Nascimento F, Fernandes da Costa C, Resende PC, Pauvolid-Correa A, Siqueira MM, Dold C, Temperton N, Dong T, Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M, Belij-Rammerstorfer S, Gilbert SC, Malik T, Carroll MW, Klenerman P, Barnes E, Dunachie SJ, Baillie V, Serafin N, Ditse Z, Da Silva K, Paterson NG, Williams MA, Hall DR, Madhi S, Nunes MC, Goulder P, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. 2021. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 184:4220–4236.e13. https://doi.org/10.1016/j.cell.2021.06.020.