BRIEF REPORT

Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors

Chee-Wah Tan, Ph.D., Wan-Ni Chia, Ph.D., Barnaby E. Young, M.R.C.P., Feng Zhu, Ph.D., Beng-Lee Lim, M.Sc., Wan-Rong Sia, B.S., Tun-Linn Thein, M.P.H., Mark I.-C. Chen, Ph.D., Yee-Sin Leo, F.R.C.P., David C. Lye, F.R.C.P., and Lin-Fa Wang, Ph.D.

SUMMARY

Emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern pose a challenge to the effectiveness of current vaccines. A vaccine that could prevent infection caused by known and future variants of concern as well as infection with pre-emergent sarbecoviruses (i.e., those with potential to cause disease in humans in the future) would be ideal. Here we provide data showing that potent cross-clade pan-sarbecovirus neutralizing antibodies are induced in survivors of severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) infection who have been immunized with the BNT162b2 messenger RNA (mRNA) vaccine. The antibodies are high-level and broad-spectrum, capable of neutralizing not only known variants of concern but also sarbecoviruses that have been identified in bats and pangolins and that have the potential to cause human infection. These findings show the feasibility of a pan-sarbecovirus vaccine strategy. (Funded by the Singapore National Research Foundation and National Medical Research Council.)

From the Programme in Emerging Infectious Diseases, Duke–NUS (National University of Singapore) Medical School (C.-W.T., W.-N.C., F.Z., B.-L.L., W.-R.S., L.-F.W.), the National Centre for Infectious Diseases (B.E.Y., T.-L.T., M.I.-C.C., Y.-S.L., D.C.L.), Tan Tock Seng Hospital (B.E.Y., M.I.-C.C., Y.-S.L., D.C.L.), Lee Kong Chian School of Medicine, Nanyang Technological University (B.E.Y., Y.-S.L., D.C.L.), Yong Loo Lin School of Medicine (Y.-S.L., D.C.L.) and Saw Swee Hock School of Public Health (Y.-S.L.), National University of Singapore, and SingHealth Duke–NUS Global Health Institute (L.-F.W.) — all in Singapore. Address reprint requests to Dr. Wang at linfa.wang@duke-nus.edu.sg or to Dr. Lye at david_lye@ncid.sg.

Drs. Tan and Chia contributed equally to this article.

This article was published on August 18, 2021, at NEJM.org.

DOI: 10.1056/NEJMoa2108453

THE CORONAVIRUS DISEASE 2019 (COVID-19) PANDEMIC THAT STARTED IN December 2019 is caused by SARS-CoV-2.[1] SARS-CoV-2 shares an overall genome sequence identity of approximately 80% with SARS-CoV (referred to here as SARS-CoV-1 to avoid confusion). SARS-CoV-1 was responsible for the SARS outbreak in 2002–2003, which included more than 8000 infections and more than 700 deaths worldwide.[2]

SARS-CoV-1 and SARS-CoV-2 belong to the species SARS-related coronavirus (subgenus sarbecovirus, genus betacoronavirus).[3] Antigenically, the two coronaviruses are placed in two distinct phylogenetic clades[1,4]; convalescent serum specimens from patients with SARS or Covid-19 lack cross-neutralization,[5] despite the majority of survivors of SARS-CoV-1 infection continuing to have detectable neutralizing antibodies against the homologous SARS-CoV-1 virus 17 years after infection.[5]

In this study, we investigated the possibility of a cross-clade boost of broad-spectrum neutralizing antibodies in survivors of SARS-CoV-1 infection in Singapore who had received the BNT162b2 mRNA vaccine (Pfizer–BioNTech) against SARS-CoV-2.

METHODS

SERUM SPECIMENS

Five serum panels were included in this study. The SARS-CoV-1–patient panel consisted of serum specimens obtained from 10 SARS-CoV-1 infection survivors in Singa-

pore at different time points (2012 and 2020) before the vaccination program started in January 2021. The SARS-CoV-2–patient panel consisted of 10 serum specimens obtained from patients with SARS-CoV-2 infection during 2020 as part of a national longitudinal study. The healthy–vaccinated panel consisted of 10 serum specimens obtained at day 14 after the second dose of the BNT162b2 mRNA vaccine, which is equivalent to 35 days after the first dose. The SARS-CoV-2–vaccinated panel consisted of 10 serum specimens obtained from Covid-19 survivors who had received two doses of BNT162b2 vaccine. The SARS-CoV-1–vaccinated panel consisted of 8 serum specimens obtained from SARS-CoV-1 infection survivors 21 to 62 days after the first BNT162b2 vaccination; 4 of the 8 specimens were obtained from patients whose serum was in the SARS-CoV-1–patient panel. Written informed consent was obtained from all patients whose serum was included in the study, and ethics approval was obtained from the National Healthcare Group and the National University of Singapore. The authors vouch for the accuracy and completeness of the data presented in this report.

SURROGATE VIRUS NEUTRALIZATION TESTS

Two different methods of performing surrogate virus neutralization tests (sVNTs) were used in this study. The singleplex sVNTs for SARS-CoV-1 and SARS-CoV-2 have been described previously[6] and are briefly described in the Methods section of the Supplementary Appendix, available with the full text of this article at NEJM.org. The sVNT kit for SARS-CoV-2 has been commercialized under the trade name cPass (GenScript), with Food and Drug Administration emergency use authorization granted in November 2020.

For multiplex sVNTs, we adapted the sVNT using the Luminex platform as described previously.[7] AviTag-biotinylated receptor-binding domain (RBD) proteins from 10 different sarbecoviruses were coated on MagPlex-Avidin microspheres (Luminex) at 5 μg per 1 million beads (see the Methods section of the Supplementary Appendix). RBD-coated microspheres (600 beads per antigen) were preincubated with serum at a final dilution of 1:20 or greater for 1 hour at 37°C with 800 rpm agitation. After 1 hour of incubation, 50 μl of phycoerythrin (PE)–conjugated human angiotensin-converting enzyme 2 (ACE2) (hACE2; 1 μg per milliliter; GenScript) was added to the well and incubated for 30 minutes at 37°C with agitation, followed by two washes with 1% bovine serum albumin in phosphate-buffered saline (PBS). The final readings were acquired with the use of the MAGPIX system (Luminex).

B-CELL PROFILING

For flow-cytometry analysis, cryopreserved peripheral blood mononuclear cells were thawed and surface stained for SARS-CoV-1–specific and SARS-CoV-2–specific B cells with the use of RBD bait tetramers (see the Methods section of the Supplementary Appendix). In brief, thawed peripheral blood mononuclear cells were incubated for 40 minutes at room temperature with SARS-CoV-1 RBD tetramers and SARS-CoV-2 RBD tetramers with 10% fetal bovine serum (FBS) in fluorescence-activated cell sorting (FACS) staining buffer (PBS supplemented with EDTA [2 mmol per liter] and 2% FBS), after which staining with surface panel fluorochrome-conjugated antibodies was performed. Surface staining was performed with viability dye (LIVE/DEAD Fixable Aqua Dead Cell Stain [Invitrogen]), anti–human CD3 antibody conjugated with fluorescein isothiocyanate (FITC), anti–human CD14 antibody conjugated with FITC, anti–human CD56 antibody conjugated with FITC, anti–human CD19 antibody conjugated with PE-Cy5, anti–human CD27 antibody conjugated with APC-H7, and anti–human CD38 antibody conjugated with BV786 for 30 minutes in FACS staining buffer at 4°C. Stained cells were washed twice with FACS staining buffer and acquired on the same day. Samples were acquired on a BD LSRFortessa analyzer or BD FACSAria III (BD Biosciences) equipped with 355-nm, 405-nm, 488-nm, 561-nm, and 640-nm lasers. SARS-CoV-1–specific and SARS-CoV-2–specific B cells were quantified by gating on CD19+ B cells after excluding Aqua-positive (dead) cells and CD3+, CD14+, and CD56+ cells.

STATISTICAL ANALYSIS

Data processing and analysis were performed with R software, version 4.0.2 (R Project for Statistical Computing) with the Tidyverse package, version 1.3.0. Continuous variables were compared between the reference group and the comparison group with the use of Wilcoxon signed-rank tests. All tests were two-sided, and a P value of less than 0.05 was considered to indicate statistical significance. Box plots and scatterplots were generated with the ggplot2 package in R soft-

ware, version 3.3.2. A binomial linear model was fitted to examine the relationship between the inhibition of ACE2–RBD interaction and pan-sarbecovirus monoclonal antibody concentration.

RESULTS

BOOSTING OF NEUTRALIZING ANTIBODIES AGAINST SARS-COV-1 AFTER SARS-COV-2 VACCINATION

To examine whether persons who have previously been exposed to sarbecovirus in one clade can have boosting of broad pan-sarbecovirus neutralizing antibodies through vaccination with the spike (S) protein of sarbecovirus from a different clade, we assessed eight survivors of SARS-CoV-1 infection in Singapore for neutralizing antibodies against both SARS-CoV-1 and SARS-CoV-2.

For quantitative comparisons of neutralizing antibodies, we used the sVNT platform our group had previously developed, which has good concordance with the live-virus neutralization test.[6,8] Before vaccination, survivors of SARS-CoV-1 infection had detectable neutralizing antibodies against SARS-CoV-1 but no or only low levels of anti–SARS-CoV-2 neutralizing antibodies (Table S1 in the Supplementary Appendix). After receiving two doses of the BNT162b2 mRNA vaccine, all eight participants had high levels of neutralizing antibodies against both SARS-CoV-1 and SARS-CoV-2. Participants 1 and 4, whose serum samples had been obtained after receipt of a single dose of mRNA vaccine, showed saturation (100%) inhibition against SARS-CoV-2, a level similar to that in the other six participants, who had received two doses. A uniform boosting of anti–SARS-CoV-1 neutralizing antibodies was also observed, regardless of the baseline level before vaccination.

ANTIBODY CROSS-NEUTRALIZATION AGAINST DIFFERENT SARBECOVIRUSES

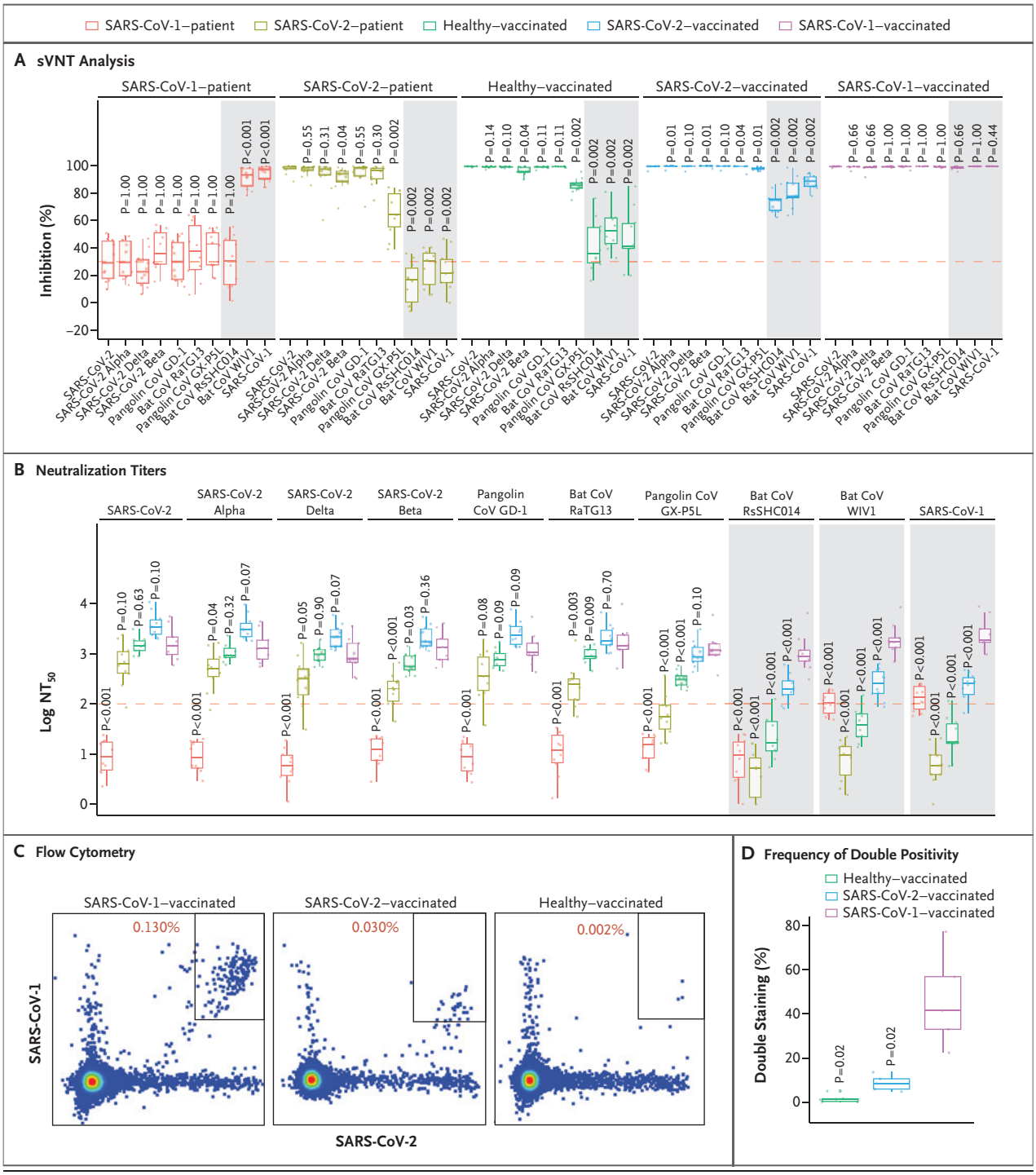
We then examined the breadth of antibody cross-neutralization against 10 different sarbecoviruses — 7 from the SARS-CoV-2 clade (the original strain of SARS-CoV-2; SARS-CoV-2 variants of concern B.1.1.7 [or alpha],[9] B.1.351 [or beta],[10] and B.1.617.2 [or delta][11]; bat coronavirus RaTG13[1]; and pangolin coronaviruses GD-1[12] and GX-P5L[12]) and 3 from the SARS-CoV-1 clade (SARS-CoV-1, bat WIV1,[13] and bat RsSHC014[13]). These viruses were chosen on the basis of their broad representation of the known sarbecovirus RBD phylogenetic spectrum (Fig. S1). To achieve more accurate and reproducible side-by-side comparison of neutralizing antibody levels, we modified the original sVNT by adapting it onto the Luminex platform to achieve multiplexity.

As shown in Figure 1A, the SARS-CoV-1–vaccinated serum panel was the only group with a broad spectrum of neutralizing antibodies against all 10 sarbecoviruses, whereas the other four serum panels showed a clear gradient of neutralizing antibody levels, with the highest targeting the homologous virus of exposure or vaccination. Because the assay was performed with a fixed dilution of 1:20 for all serum samples, the SARS-CoV-1–vaccinated panel showed a near saturation neutralization for all samples against the 10 viruses.

To better compare the neutralizing antibody titers for the different serum panels, the 50% neutralization titer was determined by serial dilution (Fig. 1B). This analysis confirmed that the SARS-CoV-1–vaccinated panel was the only one showing true pan-sarbecovirus neutralizing antibodies against all viruses examined in this study. Although the SARS-CoV-2–vaccinated panel showed enhanced levels of neutralizing antibodies against viruses in the SARS-CoV-2 clade, the neutralizing antibody level against viruses in the SARS-CoV-1 clade was still significantly lower than it was in the SARS-CoV-1–vaccinated panel.

EFFECT OF CROSS-CLADE PRIME AND BOOST

We hypothesized that the pan-sarbecovirus neutralizing antibodies observed in the SARS-CoV-1–vaccinated serum specimens were a result of enrichment of cross-reacting neutralizing antibodies from a cross-clade prime (infection) and boost (vaccination). To test this hypothesis, we used two different approaches. First, we conducted a competition study using monoclonal antibody 5B7D7, which is known to bind to all 10 RBDs and is capable of neutralizing all 10 sarbecoviruses (Fig. S2A). The data shown in Fig. S2B clearly indicate that the SARS-CoV-1–vaccinated panel is the only group of serum specimens that maintained a high level of inhibition against all viruses, confirming the findings shown in Figure 1A and 1B. In addition, the boosting of cross-reacting neutralizing antibodies in the SARS-CoV-1–vaccinated serum specimens was further confirmed by direct staining of B cells with the use of virus-specific RBD proteins. As shown in Figure 1C and 1D, the double-stained B cells (i.e., B cells that bind



RBDs from both SARS-CoV-1 and SARS-CoV-2) were significantly enriched in the SARS-CoV-1–vaccinated panel as compared with both the healthy–vaccinated and SARS-CoV-2–vaccinated panels.

DISCUSSION

Within the past two decades, we have had three major human infectious disease outbreaks caused by zoonotic coronaviruses — SARS in 2002–2003,

Figure 1 (facing page). Boosting of Cross-Clade Pan-Sarbecovirus Neutralizing Antibodies.

Panel A shows the multiplex surrogate virus neutralization test (sVNT) analysis of five panels of human serum specimens against 10 different sarbecoviruses. All serum was used at a dilution of 1:20. A cutoff of 30% was set as previously determined. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was used as the reference for comparison. The serum panels were as follows: SARS-CoV-1–patient, serum specimens from survivors of SARS-CoV-1 infection; SARS-CoV-2–patient, serum specimens obtained during 2020 from patients with SARS-CoV-2 infection; healthy–vaccinated, serum specimens obtained from healthy persons at day 14 after the second dose of the BNT162b2 messenger RNA vaccine; SARS-CoV-2–vaccinated, serum specimens obtained from Covid-19 survivors who had received two doses of BNT162b2 vaccine; and SARS-CoV-1–vaccinated, serum specimens obtained from SARS-CoV-1 infection survivors 21 to 62 days after the first dose of BNT162b2 vaccine. Panel B shows titration of neutralizing antibodies expressed as the 50% neutralization titer (NT_{50}) with the use of the same serum panels and viruses as in Panel A. Specimens were tested at dilutions from 1:20 to 1:20,480 by means of serial titration. The SARS-CoV-1–vaccinated panel was used as the reference group. A cutoff of 1:100 is indicated by the dashed line. Panel C shows representative flow cytometry plots for the SARS-CoV-1–vaccinated panel (5 specimens), the healthy–vaccinated panel (6 specimens), and the SARS-CoV-2–vaccinated panel (5 specimens), indicating the frequency of cells positive for both SARS-CoV-1 and SARS-CoV-2. Panel D shows a plot of the frequency of cells positive for both SARS-CoV-1 and SARS-CoV-2 among all SARS-CoV-1–positive or SARS-CoV-2–positive cells. The SARS-CoV-1–vaccinated panel was used as the reference group. Box plots (Panels A, B, and D) show all data points; the whiskers indicate the range, the top and bottom of each box the 75th and 25th percentiles, and the horizontal line inside each box the 50th percentile. Significance was determined with a Wilcoxon signed-rank test. P values are indicated above each plot. A P value of less than 0.05 was considered to indicate statistical significance. Viruses from the SARS-CoV-1 clade are shaded in gray in Panels A and B.

Middle East respiratory syndrome (MERS) since 2012, and Covid-19 since December 2019.[14] All three outbreaks caused devastating human and economic losses worldwide. For SARS-CoV-1 and MERS coronavirus, we still do not have a licensed vaccine. For SARS-CoV-2, the unprecedented speed of vaccine development has resulted in several licensed vaccines for mass vaccination in humans.[15]

The recent emergence of SARS-CoV-2 variants that can partially evade the immune response to vaccines that are based on the original virus strain[16-18] heightens the need for a second-generation coronavirus vaccine that will be protective against infection by all known and any future SARS-CoV-2 variants. Efforts are in progress to develop such a vaccine.[19]

However, a "dream" vaccine would cover not only SARS-CoV-2 and its known variants of concern but also future variants of concern and other coronaviruses with known potential to cause severe human diseases in the future,[20] most likely from the genus betacoronavirus.[3] Although calls have been made for developing pan-betacoronavirus or pan-coronavirus vaccines,[20] a more realistic and urgent goal is for a pan-sarbecovirus vaccine or a third-generation coronavirus vaccine. First, given their high transmissibility in humans, the sarbecoviruses pose a higher risk than the other, non-sarbecovirus zoonotic coronaviruses, despite the fact that MERS has the highest case fatality rate among the illnesses caused by these viruses.[14] Second, all zoonotic sarbecoviruses identified to date use hACE2 as the entry receptor, which ensures a higher chance of success in generating a pan-sarbecovirus vaccine by inducing cross-neutralizing antibodies that block the common hACE2–virus interaction. A deep mutagenesis study of various sarbecovirus RBDs indicated mutational constraints on folding and ACE2 binding.[4] Such constraints may explain the existence of highly conserved — but not immunodominant — cross-clade neutralizing epitopes in sarbecovirus RBDs. This finding also points to a potential pan-sarbecovirus vaccine candidate that will induce strong cross-clade neutralizing antibodies targeting the more conserved epitopes that play a role in virus neutralization, whether they are located within or outside the direct RBD–ACE2 interface.[4]

The findings from the current study showed the efficient induction of high-level and broad-spectrum pan-sarbecovirus neutralizing antibodies that can neutralize all variants of concern and five pre-emergent sarbecoviruses. Our findings showed the feasibility of achieving pan-sarbecovirus neutralization through cross-clade boosting. Previous studies have provided proof-of-concept data for a pan-sarbecovirus vaccine in studies in animals.[21-23] In comparison, our study showed boosting of pan-sarbecovirus neutralizing antibodies in humans that was more uniform

and stronger than that observed in animals. Such a high level of pan-sarbecovirus neutralizing antibody boosting may be achievable with a single-dose cross-clade boosting vaccination, as indicated with the serum from two of the eight participants in our study.

The newly developed multiplex sVNT used in this study can play a pivotal role in studies that require accurate side-by-side comparison of neutralizing antibody levels against different viruses. This is especially important when not all live viruses are available, as is the case for bat coronavirus RaTG13,[1] bat coronaviruses WIV1 and RsSHC014,[13] and pangolin coronaviruses GD-1 and GX-P5L.[12] Although we are aware that neutralizing antibodies targeting non-RBD regions do exist,[24] multiple studies have shown that for sarbecoviruses, the majority of neutralizing antibodies target the immunodominant RBD.[4,25] Previous studies have indicated that the RBD-based sVNT has a high concordance with live virus–based neutralization tests.[5,8] We have provided further data on the concordance between the sVNT and a pseudotyped virus neutralization test for three selected sarbecoviruses (see the Supplementary Appendix).

The current data in humans were obtained from SARS-CoV-1 infection survivors immunized with a SARS-CoV-2 S-based mRNA vaccine. Further investigation is needed to address whether serial administration of vaccines based on two distantly related S or RBD proteins in the reverse order — that is, priming from the SARS-CoV-2 clade followed by boosting from the SARS-CoV-1 clade — will produce a similar level of pan-sarbecovirus neutralizing antibodies. If successful, this will lay a strong foundation for the development of a third-generation Covid-19 vaccine for controlling current and emerging variants of concern, as well as for preventing future sarbecovirus pandemics.

Supported by grants from the Singapore National Research Foundation (NRF2016NRF-NSFC002-013) and National Medical Research Council (STPRG-FY19-001, COVID19RF-001, COVID19RF-003, COVID19RF-008, MOH-000535/MOH-OFYIRG19nov-0002, and RIE2020 CCGSFPOR20002).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Xin Mei Ong, Pei San Kong, Jinyan Zhang, Wharton Chan, Vivian Chen, Ying Ding, Xijian Qin, and Nelson Wang for providing logistic, technical, and reagent support and critical reading of the manuscript and all the volunteers, especially the SARS-CoV-1 infection survivors, for their contribution to this study.

REFERENCES

1. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.
2. Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med 2004;10:12 Suppl:S88-S97.
3. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536-44.
4. Starr TN, Greaney AJ, Hilton SK, et al. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell 2020;182(5):1295-1310.e20.
5. Anderson DE, Tan CW, Chia WN, et al. Lack of cross-neutralization by SARS patient sera towards SARS-CoV-2. Emerg Microbes Infect 2020;9:900-2.
6. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol 2020;38:1073-8.
7. Bossart KN, McEachern JA, Hickey AC, et al. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. J Virol Methods 2007;142:29-40.
8. Perera RAPM, Ko R, Tsang OTY, et al. Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and hamster sera. J Clin Microbiol 2021;59(2): e02504-e02520.
9. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372(6538):eabg3055.
10. Cele S, Gazy I, Jackson L, et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. Nature 2021; 593:142-6.
11. Singh J, Rahman SA, Ehtesham NZ, Hira S, Hasnain SE. SARS-CoV-2 variants of concern are emerging in India. Nat Med 2021;27:1131-3.
12. Lam TTY, Jia N, Zhang YW, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 2020;583: 282-5.
13. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 2013;503:535-8.
14. Frutos R, Serra-Cobo J, Pinault L, Lopez Roig M, Devaux CA. Emergence of bat-related betacoronaviruses: hazard and risks. Front Microbiol 2021;12:591535.
15. Fauci AS. The story behind COVID-19 vaccines. Science 2021;372:109.
16. Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130-5.
17. Wang P, Casner RG, Nair MS, et al. Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. Cell Host Microbe 2021;29(5):747-751.e4.
18. Jangra S, Ye C, Rathnasinghe R, et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. Lancet Microbe 2021;2(7):e283-e284.
19. Wu K, Choi A, Koch M, et al. Variant SARS-CoV-2 mRNA vaccines confer broad neutralization as primary or booster series in mice. April 13, 2021 (https://www.biorxiv.org/content/10.1101/2021.04.13.439482v1). preprint.
20. Cohen J. The dream vaccine. Science 2021;372:227-31.
21. Hauser BM, Sangesland M, Lam EC, et al. Engineered receptor binding domain immunogens elicit pan-coronavirus neutralizing antibodies. December 8, 2020 (https://www.biorxiv.org/content/10.1101/2020.12.07.415216v1). preprint.
22. Martinez DR, Schäfer A, Leist SR, et

al. Chimeric spike mRNA vaccines protect against Sarbecovirus challenge in mice. May 11, 2021 (https://www.biorxiv.org/content/10.1101/2021.03.11.434872v2). preprint.
23. Saunders KO, Lee E, Parks R, et al. Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses. Nature 2021;594:553-9.
24. Brouwer PJM, Caniels TG, van der Straten K, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020;369:643-50.
25. Starr TN, Greaney AJ, Addetia A, et al. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. Science 2021;371:850-4.