Emerging Microbes & Infections
2023, VOL. 12, 2271089 (7 pages)
https://doi.org/10.1080/22221751.2023.2271089

EMERGING AND RE-EMERGING CORONAVIRUSES

OPEN ACCESS Check for updates

Less neutralization evasion of SARS-CoV-2 BA.2.86 than XBB sublineages and CH.1.1

Yanping Hu[a]#, Jing Zou[a]#, Chaitanya Kurhade[a], Xiangxue Deng[b], Hope C. Chang[c], Debora K. Kim[c], Pei-Yong Shi[a], Ping Ren[c] and Xuping Xie[a,d]

[a]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, USA; [b]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA; [c]Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA; [d]Sealy Institute for Drug Discovery, University of Texas Medical Branch, Galveston, TX, USA

ABSTRACT

The highly mutated BA.2.86, with over 30 spike protein mutations in comparison to Omicron BA.2 and XBB.1.5 variants, has raised concerns about its potential to evade COVID-19 vaccination or prior SARS-CoV-2 infection-elicited immunity. In this study, we employ a live SARS-CoV-2 neutralization assay to compare the neutralization evasion ability of BA.2.86 with other emerged SARS-CoV-2 subvariants, including BA.2-derived CH.1.1, Delta-Omicron recombinant XBC.1.6, and XBB descendants XBB.1.5, XBB.1.16, XBB.2.3, EG.5.1 and FL.1.5.1. Our results show that BA.2.86 is less neutralization evasive than XBB sublineages. XBB descendants XBB.1.16, EG.5.1, and FL.1.5.1 continue to significantly evade neutralization induced by the parental COVID-19 mRNA vaccine and a BA.5 Bivalent booster. Notably, when compared to XBB.1.5, the more recent XBB descendants, particularly EG.5.1, display increased resistance to neutralization. Among all the tested variants, CH.1.1 exhibits the greatest neutralization evasion. In contrast, XBC.1.6 shows a slight reduction but remains comparably sensitive to neutralization when compared to BA.5. Furthermore, a recent XBB.1.5-breakthrough infection significantly enhances the breadth and potency of cross-neutralization. These findings reinforce the expectation that the upcoming XBB.1.5 mRNA vaccine would likely boost the neutralization of currently circulating variants, while also underscoring the critical importance of ongoing surveillance to monitor the evolution and immune evasion potential of SARS-CoV-2 variants.

ARTICLE HISTORY Received 14 September 2023; Revised 9 October 2023; Accepted 11 October 2023

Keywords SARS-CoV-2; neutralization; variants; BA.2.86; mRNA vaccine

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 pandemic, continues evolving and diverging regionally and globally. Since its initial outbreak in December 2019 [1], SARS-CoV-2 has constantly acquired genetic changes for improved fitness and immune evasion. Such adaptations have given rise to variants including Alpha, Beta, Delta, and Omicron, each of which has caused surges in infections worldwide [2]. The emergence of Omicron represents a significant shift in the evolution trajectory of SARS-CoV-2. Omicron carries more than 30 amino acid changes in its spike compared to non-Omicron variants, which account for its remarkable transmissibility and immune evasion [3–6]. Since its first detection in December 2021 [7], Omicron has rapidly become dominating and subsequently evolving into a swarm of Omicron subvariants [8]. Several Omicron sublineages, such as BA.2, BA.5, and XBB, have caused waves of infections globally. Among these sublineages, the XBB variant, a recombination of BA.2.10 and BA.2.75 sublineages, has displayed significant immune evasion [9–14] and rapidly outcompeted the previously dominant BA.5 variant since early 2023. As a result, the Food and Drug Administration (FDA) recommends the use of a monovalent XBB sublineage as the vaccine composition for the coming year 2023-2024 [15]. Currently, several other descendants of XBB, including XBB.1.16, XBB.2.3, EG.5, and FL.1.5.1, are now overriding the XBB.1.5 dominance on a global scale. As of Sep 2, 2023, EG.5, XBB.1.16, XBB.2.3, and FL.1.5.1 accounted for 22.2%, 8.3%, 9.1%, and 8.3% of the total SARS-CoV-2 infection in the United States, respectively (https://covid.cdc.gov/covid-data-tracker).

CONTACT Ping Ren peren@utmb.edu Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA; Xuping Xie xuxie@utmb.edu Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, USA; Sealy Institute for Drug Discovery, University of Texas Medical Branch, Galveston, TX, USA
#Y.H. and J.Z. made equal contributions to the study.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/22221751.2023.2271089.

More recently, a subvariant derived from BA.2, known as BA.2.86, has raised concerns about its potential to evade immunity induced by COVID-19 vaccination or prior natural infections. BA.2.86 carries over 30 amino acid changes in its spike when compared to both BA.2 and XBB.1.5, a similar magnitude of amino acid variation as previously observed between the BA.1 and Delta variants. The World Health Organization designated it as a variant under monitoring on 17 August 2023. As of September 5, 2023, there have been 41 reported sequences of BA.2.86 from 12 countries in the GISAID database, which is likely underestimated due to limited sampling. Additionally, two other circulating non-XBB Omicron subvariants CH.1.1 (a descendant of BA.2.75 sublineage) and XBC.1.6 (a recombination of Delta and Omicron sublineage BA.2) have also drawn attention due to their great immune evasion capabilities [13, 16, 17]. As of August 29, 2023, CH.1.1 has expanded from Southeast Asia to over 86 countries, while XBC.1.6 subvariant has steadily increased in prevalence in the Philippines and Australia (https://outbreak.info/situation-reports). Therefore, it is of utmost importance to compare the ability of these circulating variants to evade immunity established by vaccination or previous natural infections.

Materials and methods

Ethical statement

The Institutional Review Board at the University of Texas Medical Branch (UTMB) approved the use of human serum specimens in this study under protocol IRB number 20-0070. No informed consent was needed, as all the sera were residual specimens obtained from the routine standard of care. Patient information was completely deidentified from all specimens. No diagnosis or treatments were associated with the collection.

All virus-related activities were conducted within a biosafety level 3 (BSL-3) laboratory, equipped with redundant fans in the biosafety cabinets at UTMB. All personnel involved in the virus work adhered to stringent safety measures, including the use of powered air-purifying respirators (Breathe Easy, 3M), Tyvek suits, aprons, booties, and double gloves.

Cells

VeroE6 (ATCC® CRL-1586) cells were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). VeroE6 cells expressing TMPRSS2 (JCRB1819) were purchased from SEKISUI XenoTech, LLC. Both cell lines were cultured in a high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 1% penicillin/streptomycin. All cultures were maintained at 37°C with 5% CO_2. The Culture media, antibiotics, and supplements were all purchased from ThermoFisher Scientific (Waltham, MA). Cells were tested Mycoplasma negative by nested PCR and fluorescence microscopy at the Tissue Culture Core Facility (TCCF) at UTMB, with technical details available at https://www.utmb.edu/microbiology/core-facilities/tccf/services.

Human serum

Four panels of human sera samples with distinct vaccine and infection histories were collected at UTMB. Samples were obtained based on their availability since there were no patients recruited in the study. Tables S1-4 list the serum information. Samples were collected from both genders with varied ages and races or ethnicities. All sera were heat-inactivated at 56°C for 30 min prior to their use for neutralization assay.

Generation of recombinant Omicron sublineages-mNG SARS CoV-2

The sequence of SARS-CoV-2 variants can be accessed through GISAID (https://gisaid.org) with the following codes: XBB.1.5 (EPI_ISL_16292655), XBB.1.16 (EPI_ISL_17030006), XBB.2.3 (EPI_ISL_16475206), EG.5.1 (EPI_ISL_17700360), FL.1.5.1 (EPI_ISL_18224090), CH.1.1 (EPI_ISL_16907910), XBC.1.6 (EPI_ISL_18161082) and BA.2.86 (EPI_ISL_18110065). Figure S2 outlines the spike amino acid differences of each variant in comparison to the parental USA-WA1/2020. Each Recombinant mNG SARS-CoV-2 spike-variant was constructed by engineering the complete spike gene from the indicated variants into an infectious cDNA clone of mNG USA-WA1/2020 as reported previously [18, 19]. Briefly, standard overlap PCRs were conducted to introduce the spike mutations into the infectious clone of the mNG USA-WA1/2020. The full-length infectious cDNA clones were assembled through *in vitro* ligation. Using the assembled full-length cDNAs as templates, the genome-length RNAs were obtained through *in vitro* transcription. To rescue the recombinant viruses, the resulting RNA transcripts were electroporated into VeroE6-TMPRSS2 cells. At 48-72 h after electroporation, the supernatants (referred to as P0 stock) from the transfected cells were harvested. The P0 stocks were then inoculated onto fresh VeroE6 cells in T-175 flasks. About 48-72 h post-infection, supernatants were harvested and clarified by centrifugation at 1000×g for 10 min, stored at −80°C prior to use (referred to as P1

stock). Because our established fluorescent focus reduction neutralization tests were performed on VeroE6 cells, we used VeroE6 cells (instead of VeroE6-TMPRSS2) to amplify the P1 virus, ensuring assay consistency. Sanger sequencing was performed to verify the genome of the recombinant P1 stock viruses to ensure no undesired mutation. The infectivities (fluorescent focus units, FFUs) of P1 viruses were quantified using the fluorescent focus forming assay on VeroE6 cells before the neutralization test. The P1 virus stocks were used for the neutralization test.

Fluorescent focus reduction neutralization test (FFRNT)

Neutralization titres of human sera were measured by FFRNT using BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike mNG SARS-CoV-2s at BSL-3 using a previous established FFRNT protocol [20]. Briefly, VeroE6 cells per well were seeded in Greiner Bio-one™ 96-well microplates. After overnight infection at 37°C 5% CO_2, 2-fold serially diluted serum samples were mixed with each mNG SARS-CoV-2 (100-150 FFUs) at 37 °C for 1 h. Afterward, the serum-virus mixtures were used to infect the VeroE6 monolayer. One hour after infection, the inoculum was replaced with 100 µl of overlay medium containing 0.8% methylcellulose. After an additional 16 h of incubation at 37°C for 16 h, fluorescent foci in each well were scanned and counted using Cytation™ 7 (BioTek) imager. The foci in each well were normalized to the non-serum-treated controls to calculate the relative infectivities. The minimal serum dilution that suppressed >50% of fluorescent foci was defined as $FFRNT_{50}$. The FFRNTs were performed in duplication for each serum sample. The geometric mean titres (GMTs) from the duplicates were calculated and shown in Tables S1-4. $FFRNT_{50}$ of <20 was listed as 10 for plotting purposes and statistical analysis. Figures were initially generated using the GraphPad Prism (version 10) and further formatted using Adobe Illustrator.

Statistical analysis

Serum samples were collected based on their availability, and no specific statistical method was applied to determine the sample size beforehand. During data acquisition, the analysts were kept blind to serum information. In Figure 1 and Table S1-4, the $FFRNT_{50}$ for each panel was represented as the geometric mean titres (GMT) along with 95% confidence intervals (CI). The medians of age, and days of serum collection after infection or bivalent-booster were also indicated in Table S1-4. The group comparison of the neutralization titres was performed using the Wilcoxon matched-pairs signed-rank test in GraphPad Prism (version 10). The two-tailed *P* values for each two-group comparison can be found in Table S5. $P < 0.05$ indicates statistically significant.

Results

In this study, we sought to compare the neutralization of human sera after vaccination and/or prior SARS-CoV-2 infections against these newly emerged Omicron sublineages (XBB.2.3, XBB.1.16, EG.5.1, FL.1.5.1, CH.1.1, XBC.1.6, and BA.2.86). Supplementary Figure S1 outlines the phylogenetic relationship of these variants. To enable accurate neutralization measurement, we engineered the complete *spike* gene from each of these Omicron sublineages into the backbone of mNeonGreen (mNG) reporter USA-WA1/2020 SARS-CoV-2 (Supplementary Figure.S2). Compared with wild-type USA-WA1/2020 (a strain isolated in January 2020), the mNG SARS-CoV-2 has shown substantial attenuation *in vivo* due to the insertion of the *mNG* gene at open-reading-frame-7 of the viral genome [21], and be safely used within a BSL3 facility for neutralization [22].

We did not observe replication differences among all the recovered Omicron sublineage mNG SARS-CoV-2 variant-spike. The recovered recombinant viruses showed high infectivity and robust replication in VeroE6 cells. All the P1 viruses exhibited viral titres exceeding 10^7 PFU/ml. Before their use in determining the 50% fluorescent focus-reduction neutralization titres ($FFRNT_{50}$) of human sera, all recombinant viruses were verified by Sanger sequencing to ensure no undesired mutations in their genomes.

We analyzed four human serum panels with distinct vaccination and/or SARS-CoV-2 infection histories. The first panel comprised 28 sera obtained from individuals 14–32 (median 23) days post BA.5-bivalent-booster (referred to as BA.5-bivalent-booster sera); these sera were collected from September 30 to October 22, 2022 (Table S1). The second panel included 20 sera from individuals who had previously contracted SARS-CoV-2 and subsequently received a BA.5-bivalent-booster 15–31 (median 21) days ago (referred to as BA.5-bivalent-booster-infection sera). Samples collection for this panel took place from October 4–22, 2022 (Table S2). The infection history for the second panel was confirmed by positive nucleocapsid antibody detection or SARS-CoV-2 RT–PCR [10]. These infections could be likely attributable to BA.5 and earlier Omicron strains, such as BA.1, BA.2, and BA.2.12.1. Unfortunately, the precise infection time and specific genotype of the infecting strains could not be traced. The third panel comprised 42 sera collected from vaccinated individuals 15–117 days (median 47) after a breakthrough infection with XBB.1.5 variant (referred to as XBB.1.5-infection-

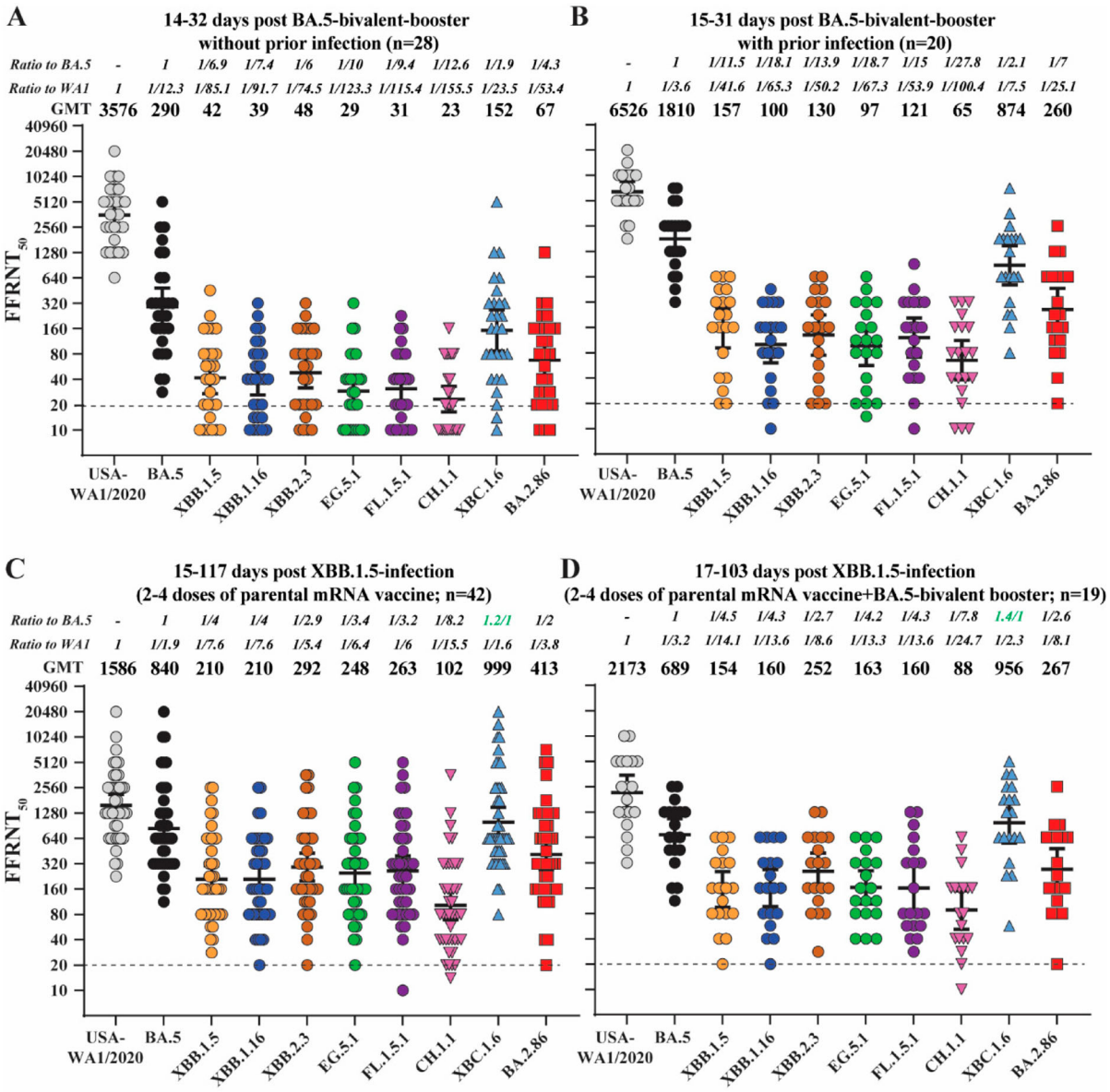

Figure 1. Neutralization titres against Omicron sublineages. (A) $FFRNT_{50}$ of 28 sera collected after BA.5-bivalent booster from individuals without prior SARS-CoV-2 infection. (B) $FFRNT_{50}$ of 20 sera collected after BA.5-bivalent-booster from individuals with prior SARS-CoV-2 infection. (C) $FFRNT_{50}$ of 42 sera collected after XBB.1.5-breakthrough infection from individuals with parental mRNA vaccination. (D) $FFRNT_{50}$ of 19 sera collected after XBB.1.5-breakthrough infection from individuals with parental mRNA vaccination plus BA.5-bivalent booster. The solid lines and numeric values above each panel indicate the geometric mean titres (GMTs). The error bars represent the 95% confidence intervals (Cis). The fold reduction in GMT against each Omicron sublineage, compared with the GMT against USA-WA1/2020 or BA.5-spike, is shown in italic font. The green italic numbers indicate more sensitivity to neutralization. The dotted line indicates the limit of detection of $FFRNT_{50}$. $FFRNT_{50}$ of <20 was treated as 10 for plotting purposes and statistical analysis. The *p* values (determined using the Wilcoxon matched-pairs signed-rank test) for group comparison of GMTs are shown in Table S5.

parental-mRNA vaccine sera). These samples were obtained between February 2 and May 23, 2023 (Table S3). The fourth panel included 19 sera collected 17–103 days (median 42) after XBB.1.5-breakthrough infection in individuals who had previously received a BA.5-bivalent booster (referred to as XBB.1.5-infection-BA.5-bivalent-plus-parental-mRNA-vaccine sera). These samples were collected between February 16 and May 18, 2023 (Table S4). For the third and fourth panels, the XBB.1.5 infection was confirmed through the sequencing of nasopharyngeal specimens; however, the infection history prior to the XBB.1.5 infection could not be determined. It is important to note that individuals in all four panels had received 2–4 doses of the parental monovalent mRNA vaccine before receiving the BA.5-bivalent-booster or experiencing the XBB.1.5-infection. Tables S1-4 summarize neutralization titres for each serum panel.

BA.5-bivalent-booster sera neutralized USA-WA1/2020-, BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6-, and BA.2.86-spike mNG SARS-CoV-2 with geometric mean titres (GMTs) of 3576, 290, 42, 39, 48, 29, 31, 23, 152 and 67, respectively (Figure 1A and Table S1). The GMTs against

BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike viruses were 12.3, 85.1, 91.7, 74.5, 123.3, 115.4, 155.5, 23.5 and 53.4-fold lower than the GMT against the USA-WA1/2020, respectively (Figure 1A). In comparison to the GMT against the BA.5-spike, the GMTs against XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike were reduced by 6.9, 7.4, 6, 10, 9.4, 12.6, 1.9, and 4.3-fold, respectively.

BA.5-bivalent-booster-infection sera neutralized USA-WA1/2020-, BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike mNG SARS-CoV-2 with GMTs of 6526, 1810, 157, 100, 130, 97, 121, 65, 874 and 260, respectively (Figure 1B and Table S2). The GMTs against BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike were 3.6, 41.6, 65.3, 50.2, 67.3, 53.9, 100.4, 7.5 and 25.1-fold lower than the GMT against the USA-WA1/2020, respectively (Figure 1B). Compared with the GMT against the BA.5-spike, the GMTs against XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6-, and BA.2.86-spike were reduced by 11.5, 18.1, 13.9, 18.7, 15, 27.8, 2.1 and 7-fold, respectively. Compared with BA.5-bivalent-booster sera without infection history, BA.5-bivalent-booster-infection sera increased the neutralizing GMTs against USA-WA1/2020, BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike by 1.8, 6.2, 3.7, 2.6, 2.7, 3.3, 3.9, 2.8, 5.8 and 3.9-fold, respectively (compare Figure 1B versus 1A).

XBB.1.5-infection-parental-mRNA vaccine sera neutralized USA-WA1/2020-, BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6- and BA.2.86-spike mNG SARS-CoV-2 with GMTs of 1586, 840, 210, 210, 292, 248, 263, 102, 999 and 413, respectively (Figure 1C and Table S3). The GMTs against BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, XBC.1.6-, and BA.2.86-spike were 1.9, 7.6, 7.6, 5.4, 6.4, 6.0, 15.5, 1.6 and 3.8-folds lower than the GMT against the USA-WA1/2020, respectively (Figure 1C). Compared with the GMT against the BA.5-spike, the GMTs against XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1-, and BA.2.86-spike were reduced by 4, 4, 2.9, 3.4, 3.2, 8.2, and 2-fold, respectively. However, the GMT against XBC.1.6-spike was slightly higher than the GMT against BA.5-spike.

XBB.1.5-infection-BA.5-bivalent-parental-mRNA-vaccine sera neutralized USA-WA1/2020-, BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3, EG.5.1-, FL.1.5.1-, CH.1.1, XBC.1.6, and BA.2.86-spike mNG SARS-CoV-2 with GMTs of 2173, 689, 154, 160, 252, 163, 160, 88, 956, and 267, respectively (Figure 1D and Table S4). The GMTs against BA.5-, XBB.1.5-, XBB.1.16-, XBB.2.3, EG.5.1-, FL.1.5.1-, CH.1.1, XBC.1.6, and BA.2.86-spike were 3.2, 14.1, 13.6, 8.6, 13.3, 13.6, 24.7, 2.3, and 8.1-fold lower than the GMT against the USA-WA1/2020-spike SARS-CoV-2, respectively (Figure 1D). In comparison to the GMT against the BA.5, the GMTs against XBB.1.5-, XBB.1.16-, XBB.2.3-, EG.5.1-, FL.1.5.1-, CH.1.1- and BA.2.86-spike were reduced by 4.5, 4.3, 2.7, 4.2, 4.3, 7.8 and 2.6-fold, respectively. Interestingly, in comparison to the fourth panel of sera, the third panel exhibited overall slightly higher neutralizing GMTs against most variants, with the exceptions being XBC.1.6 and USA-WA1/2020 (Compare Figure 1D versus 1C). These modest differences could be attributed to different immune imprinting resulting from vaccinations and/or prior SARS-CoV-2 infections [16, 23, 24], which warrants further investigations using a larger number of specimens from individuals with well-defined and documented immune backgrounds.

Discussion

Overall, our results indicate that BA.2.86 can evade serum neutralization elicited by parental and BA.5 bivalent mRNA vaccine or previous SARS-CoV-2 infections. Surprisingly, despite substantial changes in its spike protein, BA.2.86 exhibited lower immune evasion capabilities compared to XBB descendants and CH1.1. Similar observations have also been reported by several other groups using different sources of human serum samples [25, 26]. Notably, CH.1.1 displayed the greatest immune evasion among all the tested variants, indicating its potential for wider global spread. Conversely, XBC.1.6 showed the least evasion to neutralization. When compared to neutralizing GMTs against BA.5, the neutralizing GMTs against XBC.1.6 were slightly lower (less than 1-fold reduction) in individuals who had received vaccination and a BA.5-bivalent booster (Figure 1A-B) and became higher after XBB.1.5-breakthrough infection within our cohort (Figure 1C-D). Consistently, XBB descendants showed substantial evasion of neutralization elicited by parental and BA.5-bivalent mRNA vaccine or prior infections [10]. Interestingly, the more recent XBB descendants, particularly EG.5.1, exhibited more resistance to neutralization induced by vaccination and BA.5-booster or prior SARS-CoV-2 infections when compared to the earlier XBB descendant XBB.1.5, consistent with earlier reports on the increased neutralization evasion of EG.5.1 [27]. This observation aligns with the epidemiological data from earlier 2023 to the present, reflecting changes in prevalent XBB sublineages. However, when neutralization was boosted by XBB.1.5-breakthrough infection, all XBB descendants showed comparable sensitivity to neutralization. Our results

highlight that hybrid immunity, which is induced by vaccination and prior infections, can enhance the magnitude and breadth of neutralization. More importantly, XBB.1.5-breakthrough infection led to broader and more robust neutralization against the currently circulating Omicron sublineages, regardless of whether a BA.5 bivalent booster was administrated. These results reinforce the expectation that the upcoming XBB.1.5 mRNA vaccine would likely elevate the neutralization against circulating Omicron variants.

There are several limitations in our study. First, we have not analyzed cell-mediated immunity or the significance of non-neutralizing antibodies, which also play a crucial role in protecting patients from severe disease and death [28, 29]. It has previously shown that many T cell epitopes after vaccination or natural infection are preserved in Omicron spikes [30]. However, highly mutated BA.2.86 has accumulated many new spike amino acid changes. Thus, it is equally important to determine whether BA.2.86 can evade the cell-mediated immunity resulting from previous vaccination or natural infection. Second, we have not dissected the spike mutations that contribute to the observed immune evasion of the newly emerged Omicron sublineages. It's worth noting that the BA.2.86 spike sequence we used in this study contains an I670 V mutation (Figure S2), which is less common in the current BA.2.86 sequence database, has little influence in neutralization evasion of BA.2.86 [26]. It remains elusive how/what other spike mutations drive the different immune evasion between CH.1.1 and the two highly mutated BA.2.86 and XBC.1.6 (Figure S2). Third, the current results do not allow a direct comparison of neutralization from diverse immune backgrounds or baselines resulting from the vaccine type, number of vaccine doses, history of infection (when and which variants), serum collection time after previous infections, geographic and/or demographics. The complexity of immune backgrounds may explain the neutralization differences observed in serum samples collected from diverse geographic regions, as reported by other groups [25, 31]. Lastly, we did not uncover the factors that drive the emergence of BA.2.86. As suggested by our findings, aside from antibody evasion, other selection factors for higher human ACE2 binding or better viral replication or transmission may contribute to the numerous changes observed in BA.2.86. It is noteworthy that BA.2.86 has a higher binding affinity to human ACE2 [26], although the full biological significance is yet to be elucidated.

Despite these limitations, our laboratory findings would guide vaccine strategy against both current and future Omicron sublineages. Considering the co-circulation of multiple immune-evasive SARS-CoV-2 variants with distinct genetic changes, we must continue vigilantly monitoring the ongoing evolution of SARS-CoV-2 and its potential to further elude existing public health measures.

Acknowledgments

We thank many originating laboratories, who share and deposit their sequencing results timely in the GISAID database. P.-Y.S. and X.X. were supported by NIH contract HHSN272201600013C, and awards from the Sealy & Smith Foundation, the Kleberg Foundation, the John S. Dunn Foundation, the Amon G. Carter Foundation, the Summerfield Robert Foundation, and Edith and Robert Zinn. We thank the participants from whom the serum specimens were obtained. The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Declaration of interest statement

P.-Y.S. and X.X. have filed a patent on the SARS-CoV-2 reverse genetic system. Other authors declare no competing interests.

Disclosure statement

No potential conflict of interest was reported by the author (s).

Funding

This work was supported by National Institutes of Health: [grant number HHSN272201600013C]; Sealy & Smith Foundation, the Kleberg Foundation, the John S. Dunn Foundation, the Amon G. Carter Foundation, the Summerfield Robert Foundation, and Edith and Robert Zinn.

References

[1] Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–273. doi:10.1038/s41586-020-2012-7

[2] Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34:4121–4123. doi:10.1093/bioinformatics/bty407

[3] Xia H, Zou J, Kurhade C, et al. Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2. Cell Host Microbe. 2022;30:485–488.e3. doi:10.1016/j.chom.2022.02.015

[4] Willett BJ, Grove J, MacLean OA, et al. SARS-CoV-2 Omicron is an immune escape variant with an altered cell entry pathway. Nat Microbiol. 2022;7:1161–1179. doi:10.1038/s41564-022-01143-7

[5] Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature. 2022;602:671–675. doi:10.1038/s41586-021-04389-z

[6] Allen H, Tessier E, Turner C, et al. Comparative transmission of SARS-CoV-2 Omicron (B.1.1.529) and Delta (B.1.617.2) variants and the impact of

vaccination: national cohort study, England. Epidemiol Infect. 2023;151:e58, doi:10.1017/S0950268823000420
[7] Callaway E. Heavily mutated Omicron variant puts scientists on alert. Nature. 2021;600:21, doi:10.1038/d41586-021-03552-w
[8] Callaway E. COVID 'variant soup' is making winter surges hard to predict. Nature. 2022;611:213–214. doi:10.1038/d41586-022-03445-6
[9] Davis-Gardner ME, Lai L, Wali B, et al. Neutralization against BA.2.75.2, BQ.1.1, and XBB from mRNA Bivalent Booster. N Engl J Med. 2023;388:183–185. doi:10.1056/NEJMc2214293
[10] Kurhade C, Zou J, Xia H, et al. Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1 and XBB.1 by parental mRNA vaccine or a BA.5 bivalent booster. Nat Med 2023, doi:10.1038/s41591-022-02162-x
[11] Addetia A, Piccoli L, Case JB, et al. Neutralization, effector function and immune imprinting of Omicron variants. Nature. 2023, doi:10.1038/s41586-023-06487-6
[12] Zou J, Kurhade C, Patel S, et al. Neutralization of BA.4-BA.5, BA.4.6, BA.2.75.2, BQ.1.1, and XBB.1 with Bivalent Vaccine. N Engl J Med. 2023;388:854–857. doi:10.1056/NEJMc2214916
[13] Qu P, Faraone JN, Evans JP, et al. Enhanced evasion of neutralizing antibody response by Omicron XBB.1.5, CH.1.1, and CA.3.1 variants. Cell Rep. 2023;42:112443, doi:10.1016/j.celrep.2023.112443
[14] Miller J, Hachmann NP, Collier A-rY, et al. Substantial neutralization escape by SARS-CoV-2 omicron variants BQ.1.1 and XBB.1. N Engl J Med. 2023;388:662–664. doi:10.1056/NEJMc2214314
[15] Recommendation for the 2023-2024 Formula of COVID-19 vaccines in the U.S. (2023).
[16] Cao Y, Jian F, Wang J, et al. Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD evolution. Nature. 2022;614:521–529. doi:10.1038/s41586-022-05644-7
[17] Wang Q, Guo Y, Zhang RM, et al. Antibody Neutralization of Emerging SARS-CoV-2: EG.5.1 and XBC.1.6. *bioRxiv*, 2023.2008.2021.553968 (2023). doi:10.1101/2023.08.21.553968.
[18] Xie X, Lokugamage KG, Zhang X, et al. Engineering SARS-CoV-2 using a reverse genetic system. Nat Protoc. 2021;16:1761–1784. doi:10.1038/s41596-021-00491-8
[19] Xie X, Muruato A, Lokugamage KG, et al. An Infectious cDNA Clone of SARS-CoV-2. Cell Host Microbe. 2020;27:841–848. doi:10.1016/j.chom.2020.04.004
[20] Zou J, Xia H, Xie X, et al. Neutralization against Omicron SARS-CoV-2 from previous non-Omicron infection. Nat Commun. 2022;13:852), doi:10.1038/s41467-022-28544-w
[21] Johnson BA, Zhou Y, Lokugamage KG, et al. Nucleocapsid mutations in SARS-CoV-2 augment replication and pathogenesis. PLoS Pathog. 2022;18:e1010627), doi:10.1371/journal.ppat.1010627
[22] Muruato AE, Fontes-Garfias CR, Ren P, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun. 2020;11:4059), doi:10.1038/s41467-020-17892-0
[23] Wang Q, Bowen A, Tam AR, et al. SARS-CoV-2 neutralising antibodies after bivalent versus monovalent booster. Lancet Infect Dis. 2023;23:527–528. doi:10.1016/S1473-3099(23)00181-0
[24] Hoffmann M, Behrens GMN, Arora P, et al. Effect of hybrid immunity and bivalent booster vaccination on omicron sublineage neutralisation. Lancet Infect Dis. 2023;23:25–28. doi:10.1016/S1473-3099(22)00792-7
[25] Lasrado N, Collier A-RY, Hachmann NP, et al. Neutralization Escape by SARS-CoV-2 Omicron Subvariant BA.2.86. *bioRxiv*, 2023.2009.2004.556272 (2023). doi:10.1101/2023.09.04.556272.
[26] Wang Q, Guo Y, Liu L, et al. Antigenicity and receptor affinity of SARS-CoV-2 BA.2.86 spike. bioRxiv. 2023, doi:10.1101/2023.09.24.559214
[27] Zhang L, Kempf A, Nehlmeier I, et al. Neutralisation sensitivity of SARS-CoV-2 lineages EG.5.1 and XBB.2.3. Lancet Infect Dis. 2023;23:e391–e392. doi:10.1016/S1473-3099(23)00547-9
[28] Bartsch YC, Tong X, Kang J, et al. Omicron variant Spike-specific antibody binding and Fc activity are preserved in recipients of mRNA or inactivated COVID-19 vaccines. Sci Transl Med. 2022;14:eabn9243), doi:10.1126/scitranslmed.abn9243
[29] Grifoni A, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19. Cell Host Microbe. 2021;29:1076–1092. doi:10.1016/j.chom.2021.05.010
[30] Redd AD, Nardin A, Kared H, et al. Minimal Crossover between Mutations Associated with Omicron Variant of SARS-CoV-2 and CD8+T-cell epitopes identified in COVID-19 convalescent individuals. mBio. 2022;13; doi:10.1128/mbio.03617-21
[31] Yang S, Yu Y, Jian F, et al. Antigenicity and infectivity characterization of SARS-CoV-2 BA.2.86. *bioRxiv*, 2023.2009.2001.555815 (2023). doi:10.1101/2023.09.01.555815