Supplementary Appendix

Table of Contents

Materials and Methods

Ethics statement

All protocols involving specimens from human subjects recruited at Interpark Kuramochi Clinic was reviewed and approved by the Institutional Review Board of Interpark Kuramochi Clinic (approval ID: G2021-004). All human subjects provided written informed consent. All protocols for the use of human specimens were reviewed and approved by the Institutional Review Boards of The Institute of Medical Science, The University of Tokyo (approval IDs: 2021-1-0416 and 2021-18-0617).

Human serum collection

Convalescent sera were collected from fully vaccinated individuals who had been infected with BA.2 (9 2-dose vaccinated and 4 3-dose vaccinated; 11–61 days after testing. n=13 in total; average age: 45 years, range: 24–82 years, 62% male) (**Figure 1E**), and fully vaccinated individuals who had been infected with BA.5 (2 2-dose vaccinated, 17 3-dose vaccinated and 1 4-dose vaccinated; 10–23 days after testing. n=20 in total; average age: 51 years, range: 25–73 years, 45% male) (**Figure 1E**). The SARS-CoV-2 variants were identified as previously described.[1-3] Sera were inactivated at 56°C for 30 minutes and stored at –80°C until use. The details of the convalescent sera are summarized in **Table S2**.

Epidemics dynamics analysis

We modeled the epidemic dynamics of viral lineages in the USA based on the viral genomic surveillance data deposited in the GISAID database (https://www.gisaid.org/; downloaded on January 9th, 2023). In the present study, we analyzed the data from September 1, 2022. We excluded the sequence records with the following features: i) a lack of collection date information; ii) sampling in animals other than humans; iii) sampling by quarantine; iv) without the PANGO lineage information; and v) having >2% undetermined (N) nucleotide sequences. Sublineages of BQ.1.1 are summarized as BQ.1.1, and other BQ.1 sublineages are summarized as BQ.1. In addition, according to the presence or absence of S:Y144del, we classified XBB.1.5 into two groups, XBB.1.5 and XBB.1.5+ins144Y (XBB.1.5 without S:Y144del). We removed XBB from the analysis since we found that various XBB sublineages are contaminated into this category. In the downstream analysis, we only used sequences for PANGO lineages with >500 sequences in the dataset. We counted the daily frequency of each viral lineage. Subsequently, epidemic dynamics and relative R_e value for each viral lineage were estimated according to the Bayesian multinomial logistic model, described in our previous study.[2] Briefly, we estimated the logistic slope parameter β_l for each viral lineage using the model and then calculated relative R_e for each lineage r_l as $r_l = exp(\gamma\beta_l)$ where γ is the average viral generation time (2.1 days) (http://sonorouschocolate.com/covid19/index.php?title=Estimating_Generation_Time_Of_Omicr

on). For parameter estimation, the intercept and slope parameters of XBB.1 were fixed at 0. Consequently, the relative R_e of XBB.1 was fixed at 1, and those of the other lineages were estimated relative to that of XBB.1. Parameter estimation was performed via the MCMC approach implemented in CmdStan v2.31.0 (https://mc-stan.org) with CmdStanr v0.5.3 (https://mc-stan.org/cmdstanr/). Four independent MCMC chains were run with 1,000 and 4,000 steps in the warmup and sampling iterations, respectively. We confirmed that all estimated parameters showed <1.01 R-hat convergence diagnostic values and >200 effective sampling size values, indicating that the MCMC runs were successfully convergent. Information on the estimated parameters is summarized in **Table S1**. In **Figure 1A**, results for BQ.1.1, BQ.1, XBB.1, XBB.1.5, and XBB.1.5+ins144Y are shown.

Plasmid construction

Plasmids expressing the SARS-CoV-2 spike proteins of the parental D614G (B.1.1), Omicron BA.2, BA.5, BQ.1.1 and XBB.1 were prepared in our previous studies.[1,2,4-8] Plasmids expressing the spike protein of XBB.1.5 and its derivative were generated by site-directed overlap extension PCR using pC-SARS2-S XBB.1[8] as the template and the primers listed in **Table S3**. The resulting PCR fragment was subcloned into the KpnI-NotI site of the pCAGGS vector[9] using In-Fusion® HD Cloning Kit (Takara, Cat# Z9650N). Nucleotide sequences were determined by DNA sequencing services (Eurofins), and the sequence data were analyzed by Sequencher v5.1 software (Gene Codes Corporation).

Yeast surface display

Yeast surface display binding analyses for the spike receptor-binding domains of BA.2, XBB and XBB.1.5 (residues 333–527) were performed as previously described.[1-4,7,8,10-12] Cerevisiae EBY100 yeasts and pJYDC1 plasmids with RBD genes cloned between the NdeI and BamHI sites (Addgene, Cat# 162458) as previously described.[10,11] Transformed yeasts were grown in SDCAA media (220 rpm, 30°C) and expressed overnight in expression media supplemented with bilirubin (10 nM DMSO solubilized, Sigma-Aldrich, Cat# 14370) after inoculation to OD600 0.7–1.0 (220 rpm, 20°C).[10,11] Aliquots of expressed yeast cells (100 µl) were washed in ice-cold PBSB buffer (PBS with 1 mg/ml BSA) and incubated in a series of CF®640R succinimidyl ester labeled (Biotium, Cat# 92108) ACE2 peptidase domain (residues 18–740) concentrations, PBSB buffer and 1 nM bilirubin for 8 to 12 hours. After incubation, the unbound fraction was washed by ice-cold PBSB buffer and yeasts were transferred into a 96-well plate (Thermo Fisher Scientific, Cat# 268200) and 30,000 events in gated population were automatically acquired by a CytoFLEX S Flow Cytometer (Beckman Coulter, USA, Cat#. N0-V4-B2-Y4) with FITC channel data for eUnaG2 fluorescence (Abs/Em maxima 498/527 nm) and APC channel for CF640 fluorescence (Abs/Em maxima 642/662 nm) setting. Gating, analysis and fitting with nonlinear least-squares regression using Python v3.7 protocols were described previously.[1-4,7,8,10-12]

Cell culture

HEK293T cells (a human embryonic kidney cell line; ATCC CRL-3216) and HOS-ACE2/TMPRSS2 cells (kindly provided by Dr. Kenzo Tokunaga),[13,14] a derivative of HOS cells (a human osteosarcoma cell line; ATCC CRL-1543) stably expressing human ACE2 and TMPRSS2, were maintained in Dulbecco's modified Eagle's medium (DMEM) (high glucose) (Wako, Cat# 044-29765) containing 10% fetal bovine serum (FBS) (Sigma-Aldrich Cat# 172012-500ML), 100 units penicillin and 100 ug/ml streptomycin (PS) (Sigma-Aldrich, Cat# P4333-100ML).

Neutralization assay

Pseudoviruses were prepared as previously described.[1-3,5-8,12,13,15-18] Briefly, lentivirus (HIV-1)-based, luciferase-expressing reporter viruses were pseudotyped with the SARS-CoV-2 spikes. HEK293T cells (1×10^6 cells) were cotransfected with 1 μg psPAX2-IN/HiBiT,[19] 1 μg pWPI-Luc2,[19] and 500 ng plasmids expressing parental S or its derivatives using PEI Max (Polysciences, Cat# 24765-1) according to the manufacturer's protocol. Two days post transfection, the culture supernatants were harvested and centrifuged. The pseudoviruses were stored at –80°C until use. Neutralization assays were performed as previously described.[1-3,5-8,12,13,15-18] Briefly, the SARS-CoV-2 spike pseudoviruses (counting ~20,000 relative light units) were incubated with serially diluted (120-fold to 87,480-fold dilution at the final concentration) heat-inactivated sera at 37°C for 1 hour. Pseudoviruses without sera were included as controls. Then, an 40 μl mixture of pseudovirus and serum was added to HOS-ACE2/TMPRSS2 cells (10,000 cells/50 μl) in a 96-well white plate. Two days post infection, the infected cells were lysed with a Bright-Glo luciferase assay system (Promega, Cat# E2620), and the luminescent signal was measured using a GloMax explorer multimode microplate reader 3500 (Promega). The assay of each serum sample was performed in triplicate, and the 50% neutralization titer was calculated using Prism 9 (GraphPad Software).

Data availability

Dataset used in the epidemic dynamics analysis in this study is available from the GISAID database (https://www.gisaid.org; EPI_SET_230113qo). The GISAID supplemental tables for EPI_SET_230113qo is available in the GitHub repository (https://github.com/TheSatoLab/XBB.1.5_short).

Table S1. Information on estimated relative Re value for each viral lineage

PANGO lineage	Posterior mean	Posterior 2.5 percentile	Posterior 97.5 percentile	R-hat value	Effective sampling size (ESS_bulk)	Effective sampling size (ess_tail)
XBB.1.5	1.234	1.222	1.246	1.002	1952.4	4951.0
XBB.1.5+ins144Y	1.089	1.075	1.102	1.001	2894.9	7866.7
XBB.2	1.002	0.993	1.011	1.003	1729.2	4963.0
BQ.1.1	0.997	0.992	1.001	1.009	390.3	854.0
CK.1	0.996	0.987	1.005	1.003	1624.0	4448.7
CQ.2	0.991	0.981	1.001	1.002	1762.0	5079.7
BQ.1	0.979	0.975	0.983	1.009	395.6	943.6
BA.5.2.35	0.966	0.958	0.974	1.002	1484.2	4403.4
BA.2.75	0.941	0.936	0.945	1.008	436.2	1056.3
BA.5.1.27	0.939	0.934	0.945	1.004	790.9	2159.1
BA.5.2.6	0.938	0.933	0.943	1.006	577.2	1502.3
BF.14	0.933	0.926	0.940	1.002	1025.7	2773.6
BF.7.4.1	0.933	0.927	0.938	1.004	710.3	2026.7
CM.2	0.930	0.923	0.937	1.003	1191.1	3681.0
BA.5.2.34	0.927	0.921	0.932	1.005	673.0	1735.0
BF.7	0.920	0.916	0.924	1.008	435.9	1043.3
BF.11	0.917	0.912	0.922	1.006	599.0	1661.9
BE.1.1.1	0.916	0.909	0.922	1.003	933.2	2275.5
BA.5.2.23	0.915	0.909	0.921	1.004	838.2	2615.1
BF.7.4	0.915	0.910	0.920	1.006	663.8	1894.7
BA.5.1.18	0.898	0.893	0.904	1.005	714.5	1916.6
BA.5.9	0.893	0.886	0.900	1.003	1134.7	3412.8
BF.13	0.885	0.879	0.891	1.004	831.7	2318.6
BA.5.1.5	0.884	0.879	0.889	1.005	734.6	2080.0
BE.1.1	0.884	0.879	0.888	1.006	537.7	1402.8
BF.26	0.881	0.876	0.885	1.007	509.9	1284.5
BA.5.5.1	0.871	0.866	0.877	1.004	868.0	2493.4
BA.5.2.3	0.869	0.862	0.875	1.002	1198.5	3518.6
BA.5.2.20	0.866	0.861	0.871	1.005	667.4	2045.0
BA.5.1.22	0.865	0.860	0.870	1.005	718.1	2217.6
BA.5.2.31	0.865	0.858	0.871	1.003	1054.5	2983.0
BE.1.4	0.862	0.856	0.869	1.003	1173.4	3736.9
BA.5.2	0.862	0.858	0.866	1.009	393.4	862.8
BA.5.1.10	0.861	0.856	0.866	1.006	650.1	1720.5
BA.5.1.6	0.861	0.855	0.868	1.004	1108.2	2816.6
BA.5	0.861	0.857	0.865	1.007	505.0	1329.1
BA.5.2.21	0.861	0.856	0.865	1.005	595.0	1765.2
BF.10	0.859	0.855	0.863	1.008	461.5	1127.6
BA.5.1.3	0.858	0.851	0.865	1.002	1302.3	3986.9
BF.5	0.857	0.853	0.862	1.007	527.5	1417.6
BA.5.2.9	0.856	0.852	0.860	1.007	494.7	1199.0
BA.4.6.5	0.855	0.850	0.861	1.004	808.8	2293.9

(Table S1, continued)

BA.5.1	0.855	0.851	0.859	1.008	415.0	957.0
BA.5.1.23	0.855	0.849	0.860	1.004	786.8	1947.4
BA.4.6	0.854	0.850	0.858	1.008	398.5	887.0
BA.5.1.25	0.851	0.845	0.858	1.003	1203.5	3445.9
BA.5.2.1	0.850	0.847	0.854	1.009	383.5	811.7
BE.1	0.849	0.843	0.854	1.005	745.1	2051.5
BA.5.1.30	0.847	0.841	0.853	1.004	999.1	2768.6
BA.5.1.2	0.847	0.840	0.854	1.003	1193.7	3556.3
BA.5.5	0.844	0.840	0.848	1.007	505.6	1218.6
BA.5.2.22	0.843	0.836	0.850	1.003	1291.3	4064.0
BF.27	0.837	0.831	0.843	1.003	909.9	2698.9
BF.21	0.836	0.829	0.842	1.003	1081.6	3131.9
BA.5.1.1	0.831	0.825	0.836	1.005	846.8	2285.5
BF.8	0.830	0.823	0.837	1.002	1350.1	3848.4
BA.5.6	0.819	0.815	0.824	1.007	577.2	1479.4
BE.3	0.817	0.810	0.823	1.002	1237.1	3884.6
BA.4.1	0.800	0.793	0.806	1.002	1496.2	4352.3

The Re value of XBB.1 is set at 1.

Table S2. Human sera used in this study

SARS-CoV-2 variant infected	Donor ID	Sex	Age	Date of test (YYYY/MM/DD)	Date of sampling (YYYY/MM/DD)	Prior infection?	Prior vaccination?	Vaccine	Date of 1st vaccination (YYYY/MM/DD)	Date of 2nd vaccination (YYYY/MM/DD)	Date of 3rd vaccination (YYYY/MM/DD)	Date of 4rd vaccination (YYYY/MM/DD)
BA.2	P378	Male	43	2022/03/28	2022/04/10	No	Yes	BNT162b2	2021/10/10	2021/10/31		
BA.2	P398	Male	48	2022/04/13	2022/04/30	No	Yes	BNT162b2	2021/09/18	2021/10/09	2022/04/09	
BA.2	P407	Male	29	2022/05/01	2022/05/12	No	Yes	mRNA-1273	2021/09/13	2021/10/11		
BA.2	P401	Male	35	2022/04/22	2022/05/05	No	Yes	BNT162b2	2021/09/09	2021/09/30		
BA.2	P412	Female	82	2022/05/04	2022/05/26	No	Yes	BNT162b2	2021/06/11	2021/07/09		
BA.2	6449	Male	43	2022/04/03	2022/04/23	No	Yes	BNT162b2	2021/08/13	2021/09/11		
BA.2	6355	Male	50	2022/04/02	2022/04/20	No	Yes	Not applicable	2021/04/28	2021/05/19	2022/01/19	
BA.2	6547	Male	54	2022/04/06	2022/04/22	No	Yes	BNT162b2	2021/08/25	2021/09/15		
BA.2	7951	Female	71	2022/04/25	2022/05/12	No	Yes	Mix	2021/06/20 (BNT162b2)	2021/07/16 (BNT162b2)	2022/02/16 (mRNA-1273)	
BA.2	8645	Female	41	2022/05/07	2022/05/20	No	Yes	BNT162b2	2021/05/23	2021/06/13	2022/01/20	
BA.2	8682	Female	25	2022/05/08	2022/05/24	No	Yes	BNT162b2	2021/09/03	2021/09/27		
BA.2	5949	Male	24	2022/03/22	2022/05/22	No	Yes	mRNA-1273	2021/08/05	2021/09/02		
BA.2	8796	Female	34	2022/05/10	2022/06/05	No	Yes	BNT162b2	2021/09/26	2021/10/17		
BA.5	P427	Female	49	2022/07/06	2022/07/25	No	Yes	Not applicable	2021/07/30	2021/08/25	2022/03/18	
BA.5	P440	Male	25	2022/07/24	2022/08/07	No	Yes	BNT162b2	2021/11/24	2021/12/15		
BA.5	P439	Female	73	2022/07/23	2022/08/08	No	Yes	BNT162b2	2021/06/19	2021/07/20	2022/02/04 (mRNA-1273)	
BA.5	P451	Female	55	2022/07/29	2022/8/12	No	Yes	BNT162b2	2021/04/26	2021/05/20	2022/01/18	
BA.5	P456	Male	44	2022/08/04	2022/08/14	No	Yes	BNT162b2	2021/08/11	2021/09/01	2022/03/13 (mRNA-1273)	
BA.5	P455	Male	29	2022/08/03	2022/08/17	No	Yes	mRNA-1273	2021/07/26	2021/08/27	2022/04/18	
BA.5	P464	Male	63	2022/08/08	2022/08/19	No	Yes	BNT162b2	2021/08/08	2021/08/29	2022/04/07 (mRNA-1273)	
BA.5	9341	Male	56	2022/06/12	2022/06/30	No	Yes	BNT162b2	2021/08/10	2021/08/31	2022/03/18	
BA.5	9584	Male	55	2022/07/08	2022/07/25	No	Yes	BNT162b2	2021/07/14	2021/08/05	2022/03/22	
BA.5	11318	Female	51	2022/07/24	2022/08/05	No	Yes	BNT162b2	2021/09/01	2021/09/22	2022/05/19 (mRNA-1273)	
BA.5	23S-08	Male	25	2022/07/23	2022/08/08	No	Yes	BNT162b2	2021/04/27	2021/05/18	2022/01/11	
BA.5	11597	Female	41	2022/07/26	2022/08/08	No	Yes	BNT162b2	2021/04/30	2021/05/21	2022/01/11	
BA.5	10978	Female	46	2022/07/22	2022/08/11	No	Yes	BNT162b2	2021/08/27	2021/09/17	2022/05/15	
BA.5	10826	Male	63	2022/07/21	2022/08/11	No	Yes	BNT162b2	2021/07/27	2021/08/17	2022/03/04	2022/8/9 (mRNA-1273)
BA.5	11079	Female	65	2022/07/23	2022/08/11	No	Yes	mRNA-1273	2021/07/08	2021/08/05	2022/03/17	
BA.5	14847	Female	70	2022/08/13	2022/08/25	No	Yes	BNT162b2	2021/07/13	2021/08/20	2022/03/08 (mRNA-1273)	
BA.5	13180	Female	63	2022/08/04	2022/08/25	No	Yes	BNT162b2	2021/07/16	2021/08/06	2022/03/08 (mRNA-1273)	
BA.5	12912	Male	64	2022/08/02	2022/08/25	No	Yes	mRNA-1273	2021/09/02	2021/09/30	2021/04/01	
BA.5	14956	Female	33	2022/08/13	2022/08/28	No	Yes	BNT162b2	2021/09/06	2021/10/07		
BA.5	15707	Female	52	2022/08/16	2022/08/29	No	Yes	BNT162b2	2021/08/07	2021/08/28	2022/04/03	

Table S3. Primers used in this study

Primer name	Primer sequence (5'-to-3')	Use
Omicron universal Fw	cactatagggcgaattgggtaccatgtttgtgttcctggt	Preparation of S expression plasmid
BA2 Rv	agctccaccgcggtggcggccgctcaggtgtagtgcagtttca	Preparation of S expression plasmid
pC-S_XBB_S486P_Fwd	aatggagtggccggcCCCaactgttacAGCcca	Preparation of S expression plasmid
pC-S_XBB_S486P_Rev	tggGCTgtaacagttGGGgccggccactccatt	Preparation of S expression plasmid
pC-S_BA.2_F486P_Fwd	aatggagtggccggcCCCaactgttactttcca	Preparation of S expression plasmid
pC-S_BA.2_F486P_Rev	tggaaagtaacagttGGGgccggccactccatt	Preparation of S expression plasmid
pC-S_XBB_insY144_Fwd	ccattcctgGACgtcTACtacCAGaagaacaac	Preparation of S expression plasmid
pC-S_XBB_insY144_Rev	gttgttcttCTGgtaGTAgacGTCcaggaatgg	Preparation of S expression plasmid
XBB_F486P_F	CAGGCCGGTAACAAACCTTGTAATGGTGTTGCAGGTCC AAATTGTTACTCTCCTTTACAATCATATGGTTTCC	Preparation of S RBD expression plasmid

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