Neutralizing antibody evasion and receptor binding features of SARS-CoV-2 Omicron BA.2.75

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Abstract

The Omicron subvariants BA.2.75 is rapidly raising in India. BA.2.75 also shows a local growth advantage compared to BA.2.38 and BA.4/BA.5[1]. Its immune evasion capability and receptor binding affinity is unclear and requires investigation. Here, we show that BA.2.75 is more neutralization evasive than BA.2.12.1 against the plasma from post-vaccination BA.2 infection, but less compared to BA.4/BA.5. However, as shown in a small sample of plasma from post-vaccination Delta infection, BA.2.75 seems to be more immune evasive than BA.4/BA.5 in Delta-stimulated immune background, which may explain BA. 2.75's growth advantage over BA.4/BA.5 in India. The additional N460K, G446S, D339H and R493Q mutations carried by BA.2.75 allows it to escape BA.2-effective neutralizing antibodies of different RBD epitopes, and BA.2.75 has a distinct antibody escaping profile from BA.4/BA.5. Compared to BA.2, REGN10933 and COV2-2196 partially recovered neutralization against BA.2.75 due to R493Q reversion. However, the efficacy of their corresponding cocktail was not significantly changed, since REGN10987 and COV2-2130 showed reduced neutralizing activity due to G446S. BA.2.75 exhibits higher ACE2-binding affinity than BA.4/BA.5, which should be contributed by R493Q and N460K, according to deep mutational scanning (DMS) results[2]. This affinity-strengthening feature is being further examined and verified, which will be updated soon.

Main

BA.2.75 is growing rapidly, contributing to over 20% of recently reported sequences in India[1]. Compared with BA.2, BA.2.75 carries 9 substitutions on the spike, with five on the N-terminal domain (NTD), including K147E, W152R, F157L, I210V and G257S, and four on the receptor-binding domain (RBD), namely D339H, G446S, N460K and R493Q[3]. Among them, G446S appeared in BA.1, and R493Q reversion is observed in BA.4/BA.5. N460K and D339H mutations have not been observed on prevailing variants, and their functions remain unclear. Since BA.2.75 shows a significant local growth advantage compared to BA.2.38 and BA.4/BA.5[1], its humoral immune evasion capability and receptor-binding affinity need immediate evaluation.

Here, by pseudovirus neutralization assays, we show that unlike BA.2.38, BA.2.75 exhibits significantly stronger humoral immune evasion than BA.2 in plasma samples from individuals who had received 3 doses of CoronaVac prior to BA.1 or BA.2 breakthrough infection (Fig. 1a). BA.2.75 is also more neutralization evasive than BA.2.12.1 in post-vaccination BA.2 convalescents, but less compared to BA.4/BA.5[4-9]. However, BA.2.75 seems to be more immune evasive than BA.4/BA.5 in Delta-infection backgrounds, which may explain BA.2.75's growth advantage in India (Fig. 1a). This phenomenon could be contributed by the R452 stimulation of Delta, which could result in potent antibodies that are highly effective against BA.4/BA.5 (also carries R452). Noteworthy, the sample size of Delta convalescents here is small.

As for antibody therapeutics, REGN10933[10] and COV2-2196[11] partially recovered their activities against BA.2.75 due to R493Q reversion (Fig. 1b). However, REGN10987 and COV2-2130 were affected by G446S, resulting in only a mild change in the neutralizing activity of the corresponding cocktails against BA.2.75 (Fig. 1b). No other significant changes on antibody therapeutics were observed. Note that contrasting efficacy results regarding LY-CoV1404 (bebtelovimab[12]) and S309 (Sotrovimab[13]) have been reported[14], where the discrepancy may be caused by different pseudovirus

platforms (VSV versus Lentivirus).

To investigate the antibody escaping mechanism of BA.2.75, we determined the neutralizing activities of a panel of potent BA.2-effective neutralizing antibodies from five epitope groups that could be potentially affected by D339H, N460K, R493Q and G446S[4,15] (Fig. 2a-b). SARS-CoV-2 neutralizing antibody epitope groups were previously defined by unsupervised clustering based on high-throughput yeast-display based deep mutational scanning (DMS) results[4,15] (Fig. 2b).

N460K can cause a global reduction in neutralizing activities of Group A antibodies, which agrees with DMS results[4,15]. Interestingly, BA.2.75 causes heavier neutralization reduction compared to BA.2+460K, suggesting that R493Q can also induce Group A antibody escape (Fig. 2a-b). This is reasonable since all Group A antibodies tested here are isolated from individuals that were infected by BA.1, which carries R493. However, the R493Q reversion could also recover the neutralization capability of some wildtype-induced Group A antibodies, similar to that of REGN10933 and COV2-2196. Group B antibodies were not affected by BA.2.75, but notably, most of them were completely escaped by BA.4/BA.5[4] (Fig. 2a). Reduced activities against BA.2.75 were observed for antibodies in Group D1 and D2, mostly due to G446S, given that their neutralizing activities against BA.2.75 are quite similar to that against BA.1 (Fig. 2a). Note that due to L452R mutation, BA.4/BA.5 escapes the majority of D1 antibodies[4]. Lastly, D339H also causes a systematic neutralization reduction for antibodies in Group E1, which could be attributed to the change of the surface charge. Together, results suggest all four additional RBD mutations of BA.2.75 compared to BA.2 could cause neutralization escape, and the antibody evasion profile of BA.2.75 is distinct from that of BA.4/BA.5.

Determined by surface plasma resonance (SPR), BA.2.75's RBD exhibits higher hACE2-binding affinity than BA.2's (Fig. 3), as well as the RBD of BA.2.12.1 and BA.4/BA.5 as indicated from the previous results[4]. According to the DMS results[2], the increased binding affinity should be contributed by R493Q and N460K. We are confirming this affinity-strengthen feature and will update soon. The higher receptor-

binding affinity does not directly translate into higher infectivity or disease severity; however, it does indicate that more immune escaping mutations, especially those that come with a cost of ACE2 binding strength, could be incorporated into BA.2.75's RBD without heavy penalty on transmission capability.

Methods

Plasma isolation

Blood samples were obtained from SARS-CoV-2 vaccinee convalescent individuals who had been infected with Delta, BA.1, BA.2 (Supplementary Table 1). Blood samples were obtained 4 weeks after the booster shot or 4-8 weeks after discharge from the hospital after BA.1 or BA.2 or Delta infection. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Whole blood samples were diluted 1:1 with PBS+2% FBS and then subjected to Ficoll (Cytiva, 17-1440-03) gradient centrifugation. After centrifugation, plasma was collected from upper layer. Plasma samples were aliquoted and stored at −20 °C or less and were heat-inactivated before experiments.

Pseudovirus neutralization assay

SARS-CoV-2 variants Spike pseudovirus was prepared based on a vesicular stomatitis virus (VSV) pseudovirus packaging system. BA.2 pseudovirus is constructed with the following mutations based on wildtype: (GISAID: EPI_ISL_7580387) T19I, L24S, del25-27, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. BA.2.75: BA.2+K147E+W152R+F157L+I210V+G257S+D339H+G446S+N460K+R493Q.
BA.2.38: BA.2+K417T. BA.4/BA.5: T19I, L24S, del25-27, del69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. Plasmid is constructed into

pcDNA3.1 vector. G*ΔG-VSV virus (VSV G pseudotyped virus, Kerafast) and spike protein plasmid were transfected to 293T cells (American Type Culture Collection [ATCC], CRL-3216). After culture, the pseudovirus in the supernatant was harvested, filtered, aliquoted, and frozen at −80°C for further use.

Huh-7 cell line (Japanese Collection of Research Bioresources [JCRB], 0403) were used in pseudovirus neutralization assays. Plasma samples or antibodies were serially diluted in culture media and mixed with pseudovirus and incubated for 1 h in a 37°C incubator with 5% CO_2. Digested Huh-7 cells were seeded to the antibody-virus mixture. After one day culture in the incubator, the supernatant was discarded. D-luciferin reagent (PerkinElmer, 6066769) was added into the plates and incubated in darkness for 2 min and cell lysis was transferred to detection plates. The luminescence value was detected with a microplate spectrophotometer (PerkinElmer, HH3400). IC50 was determined by a four-parameter logistic regression model.

Surface plasmon resonance

SPR experiments were performed on the Biacore 8K (GE Healthcare) instrument. Human ACE2 was immobilized onto CM5 sensor chips. Purified RBD protein of SARS-CoV-2 variants were serially diluted. RBD protein solutions were injected and the response units were recorded at room temperature. The data were fitted to 1:1 binding model by using Biacore Evaluation Software (GE Healthcare).

Acknowledgments

This project is financially supported by the Ministry of Science and Technology of China (CPL-1233).

Author contributions

Y.C., X.W. and X.S.X designed the study. Y.C., F.J. and wrote the manuscript with inputs from all authors. F.J. and J.W. (BIOPIC) performed and analyzed the yeast-display-based deep mutational scanning experiments. Y.C., W.S., A.Y., Y.Y., T.X., P.W., J.W. (Changping Laboratory), S.Y., L.L., R.A., Yao W., N.Z., X.N., L.Y., C.Y.,

F.S. performed and analyzed the pseudovirus neutralization assays. Y.Y. and Youchun W. prepared the VSV-based SARS-CoV-2 pseudovirus. Q.G. proofed the manuscript. C.Y., R.F. and X.W. performed surface plasma resonance (SPR) experiments. X.H. and R.J. recruited the SARS-CoV-2 vaccinees and Omicron/Delta convalescents.

Competing interests

X.S.X. and Y.C. are founders of Singlomics Biopharmaceuticals. Other authors declare no competing interests.

Figure Legends

Fig. 1 | Humoral immune evasion caused by BA.2.38 and BA.2.75.

a, Half neutralizing titers (NT50) of plasma samples from vaccinated individuals and post-vaccination BA.1, BA.2, or Delta convalescents, against multiple SARS-CoV-2 variants measured by VSV-pseudovirus neutralization assays. Data of vaccinated individuals and BA.1 post-vaccination convalescents against D614G, BA.1, BA.2, BA.2.12.1, and BA.4/ BA.5 have been previously published[4]. **b,** Neutralizing activities of therapeutic neutralizing antibodies against SARS-CoV-2 variants and SARS-CoV-1 pseudoviruses. Antibody neutralization data of BA.2, BA.2.12.1, and BA.4/BA.5 have been previously published[4].

Fig. 2 | Antibody escaping mechanism of BA.2.75.

a, Neutralizing activities against SARS-CoV-2 variants pseudoviruses by selected potent BA.2-effective antibodies from epitope groups A, B, D1, D2 and E1. *$p<0.05$, **$p<0.01$, ***$p<0.001$. P-values were calculated using two-tailed Wilcoxon singed-rank test of paired samples. The black bar indicates geometric mean of IC50. **b,** Average site escape scores of antibodies in epitope groups A, B, D1, D2 and E1 are projected onto the structure of SARS-CoV-2 RBD (PDB: 6M0J). Escaping hotspots are annotated.

Fig. 3 | Receptor-binding affinity of BA.2.75 RBD.

SPR sensorgrams for the measurement of hACE2-binding affinities of BA.2 and BA.2.75 RBD.

Supplementary Table 1

Summarized information of SARS-CoV-2 vaccinated individuals and convalescents involved in the plasma neutralization study.

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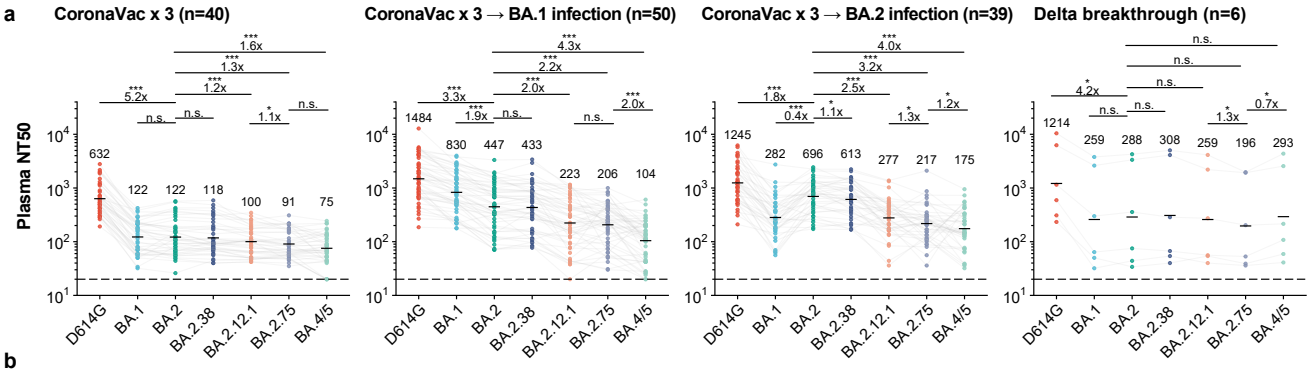
Figure 1



b

IC50 (ng/mL)	LY-CoV016	LY-CoV555	LY-CoV1404	REGN 10933	REGN 10987	COV2-2196	COV2-2130	BRII-196	BRII-198	S309	DXP-604	ADG-2	SA58 (BD55-5840)	SA55 (BD55-5514)	LY-CoV016+ LY-CoV555	REGN10933+ REGN10987	COV2-2196+ COV2-2130	BRII-196+ BRII-198	SA55+SA58
D614G	32	15	0.7	5.6	5.7	1.6	2.5	53	1239	74	11	11	0.9	11	20	5.0	2.1	81	2.1
BA.2	*	*	0.9	*	590	4312	6.3	8530	8990	918	219	*	12	7.2	*	821	8.2	8610	7.8
BA.2.12.1	*	*	0.8	*	499	5521	11	7620	*	989	201	*	5.0	5.2	*	714	18	*	5.0
BA.4/BA.5	*	*	0.9	*	520	*	23	7124	*	792	6264	*	3.9	5.0	*	709	40	*	4.5
BA.2.38	*	*	1.3	*	493	3221	11	648	7553	926	24	*	4.5	2.4	*	816	15	992	3.1
BA.2.75	*	*	2.2	278	*	225	564	1730	6622	749	5920	*	246	4.3	*	410	177	5339	6.6
SARS-CoV-1	*	*	*	*	*	*	*	*	*	31	*	1.7	5.6	4.4	*	*	*	*	4.6

<30	30~1000	>1000	* >10,000

Fig. 1 | Humoral immune evasion caused by BA.2.38 and BA.2.75

a, Half neutralizing titers (NT50) of plasma samples from vaccinated individuals and post-vaccination BA.1, BA.2, or Delta convalescents, against multiple SARS-CoV-2 variants measured by VSV-pseudovirus neutralization assays. Data of vaccinated individuals and BA.1 post-vaccination convalescents against D614G, BA.1, BA.2, BA.2.12.1, and BA.4/ BA.5 have been previously published[4]. b, Neutralizing activities of therapeutic neutralizing antibodies against SARS-CoV-2 variants and SARS-CoV-1 pseudoviruses. Antibody neutralization data of BA.2, BA.2.12.1, and BA.4/BA.5 have been previously published[4].

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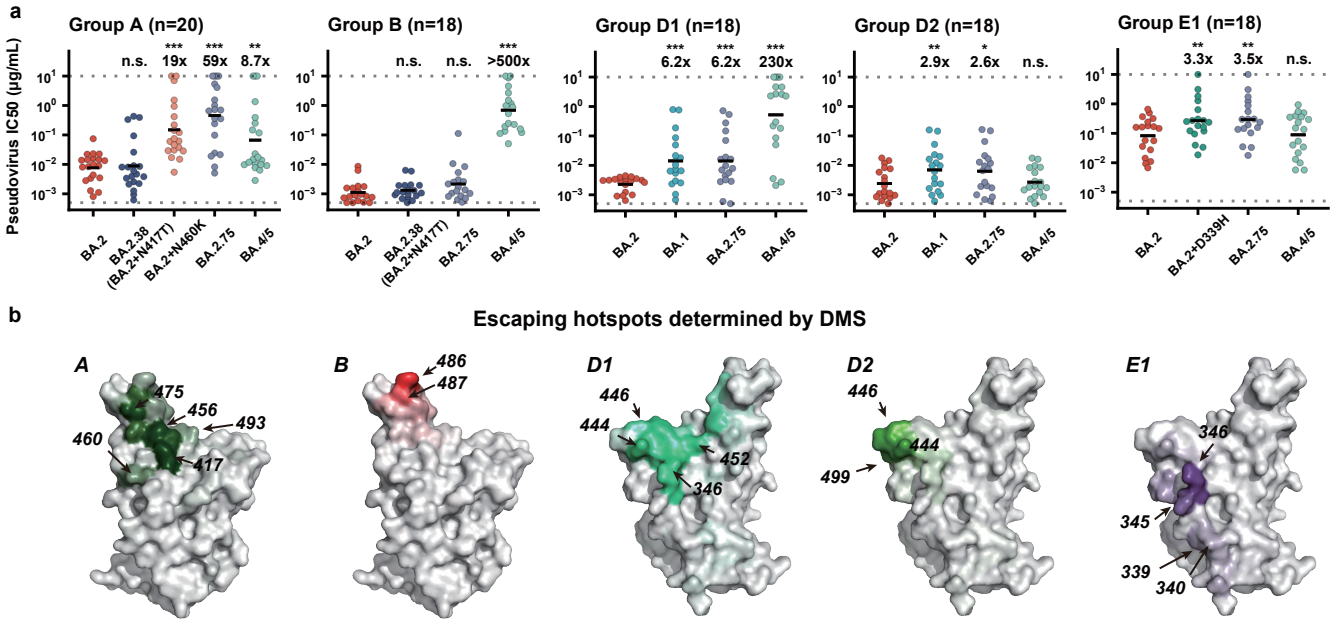

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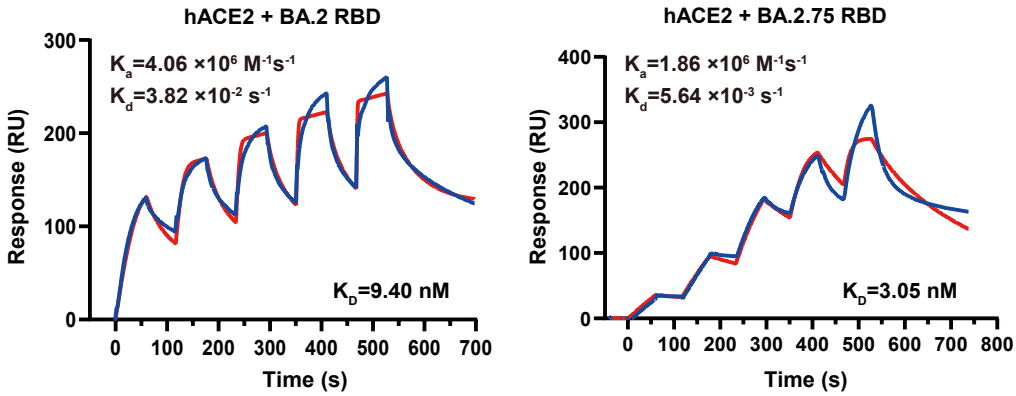

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