Antigenicity and infectivity characterisation of SARS-CoV-2 BA.2.86

The newly emerged SARS-CoV-2 saltation variant, BA.2.86, has raised global concern (appendix 1 p 7). By Sept 8, 2023, 95 sequences were detected, of which their early sequences originated from multiple countries, had no epidemiological relevance, and were found in individuals without a history of travel, suggesting the presence of underlying international transmission (appendix 1 p 7). On Aug 18, 2023, WHO designated this variant as a variant under monitoring on account of the many mutations it carries. BA.2.86 harbours numerous mutations that deviate significantly from the currently circulating strains, with 33 spike mutations and 14 receptor binding domain (RBD) mutations compared with BA.2 and 35 spike mutations and 12 RBD mutations compared with XBB.1.5 (appendix 1 p 7). Along with the shared mutations with XBB.1.5 (T19I, 24_26del, A27S, G142D, 144del, G339H, G446S, N460K, and F486P), the additional mutations I332V, K356T, V445H, N450D, N481K, A484K, and 483del on BA.2.86's RBD are likely to enhance immune evasion as previously reported.[1,2] Many unusual mutations on the N-terminal domain, such as R21T, S50L, 69_70del, V127F, F157S, R158G, 211del, L212I, L216F, H245N, and A264D, might alter the antigenicity of BA.2.86 as well. These findings underscore the potential of BA.2.86 for global spread. Therefore, an experimental assessment of the antigenicity and infectivity of BA.2.86 is urgently needed.

First, we generated the pseudovirus of BA.2.86 and established its antigenic distance from B.1 (D614G), BA.5, BQ.1.1, and XBB using serum samples from mice that had received two doses of spike mRNA vaccines (figure A; appendix 1 p 8). BA.2.86 showed a high resistance to serum neutralisation across all vaccine groups (appendix 1 p 8). Antigenic cartography calculated on the basis of the pseudovirus neutralisation titres showing that BA.2.86 was antigenically distinct from wild-type, BA.2, BA.5, and XBB.1.5, suggesting a substantial antigenic drift, which indicates that BA.2.86 could strongly evade XBB-induced antibodies (figure A).

To assess the immune evasion characteristics of BA.2.86, pseudovirus neutralisation assays were performed against XBB infection convalescent plasma and a panel of monoclonal antibodies (figure B–C). All involved participants received three doses of inactivated vaccines before having a XBB (XBB subvariants with S486P substitution) breakthrough infection. The first cohort (n=27) included individuals with single post-vaccination XBB breakthrough infection and the second cohort (n=54) comprised convalescents who had a XBB reinfection after BA.5 or BF.7 breakthrough infection (appendix 2). We found that BA.2.86 could induce significant antibody evasion of XBB-stimulated plasma (figure B). BA.2.86's immune evasion capability even exceeded EG.5 and was similar to variants with the adjacent residue flipping mutation L455F and F456L (FLip variants) such as HK.3 (XBB.1.5, L455F, and F456L).[3] Notably, the relative activity against HK.3 and BA.2.86 varied from sample to sample, indicating a large antigenic distance despite a similar amount of evasion. As for monoclonal neutralising antibody drugs, all approved antibodies were unable to neutralise BA.2.86 well, but SA55 was effective (figure C).[4] As expected, the E554K mutation carried by BA.2.86, which is located on the binding interface of SD1-targeting neutralising antibodies, could escape SD1-targeting neutralising antibodies, represented by S3H3, an antibody that is effective against many evasive mutants including the FLip variants (appendix 1 p 9).[5] Notably, we found that the E554K mutation added on XBB.1.5 could also enhance plasma evasion, suggesting that SD1-targeting neutralising antibodies compose a considerable amount of XBB-stimulated convalescent plasma (appendix 1 p 9).[6] Furthermore, we showed that by switching the RBD part of BA.2.86 to XBB.1.5, the pseudovirus still had a higher immune evasion capability than XBB.1.5 and XBB.1.5 plus E554K, suggesting that the mutations in BA.2.86's N-terminal domain could also induce significant neutralising evasion (appendix 1 p 9). To delineate the key RBD mutations of BA.2.86's enhanced immune evasion capability compared with XBB.1.5, we also tested a panel of XBB.1.5-effective neutralising antibodies against XBB.1.5-based pseudoviruses carrying single BA.2.86 RBD mutations (appendix 1 p 9).[7] Results showed that N450D, K356T, L452W, A484K, V483del, and V445H were involved in BA.2.86's enhanced immune evasion compared with XBB.1.5. Specifically, K356T, L452W, and P445H evaded the majority of antibodies in class 3 defined by their targeting epitope on RBD, and A484K and V483del contributed to the evasion of neutralising antibodies in class 2 (appendix 1 p 9). Such systematic evasion of XBB-effective neutralising antibodies explain the distinct antigenicity of BA.2.86 and its resistance to XBB convalescent plasma. Together, the aforementioned data suggest that BA.2.86 is highly immune evasive and could have advantages over currently circulating variants regarding the ability to resist XBB-induced humoral immunity.

Saltation variants might have a compromised efficiency with regards to infecting host cells to gain a strong capability of evading neutralising antibodies elicited during the antibody–virus coevolution in long-term continuous host infection.[8] Therefore, we next evaluated BA.2.86's

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See Online for appendix 1

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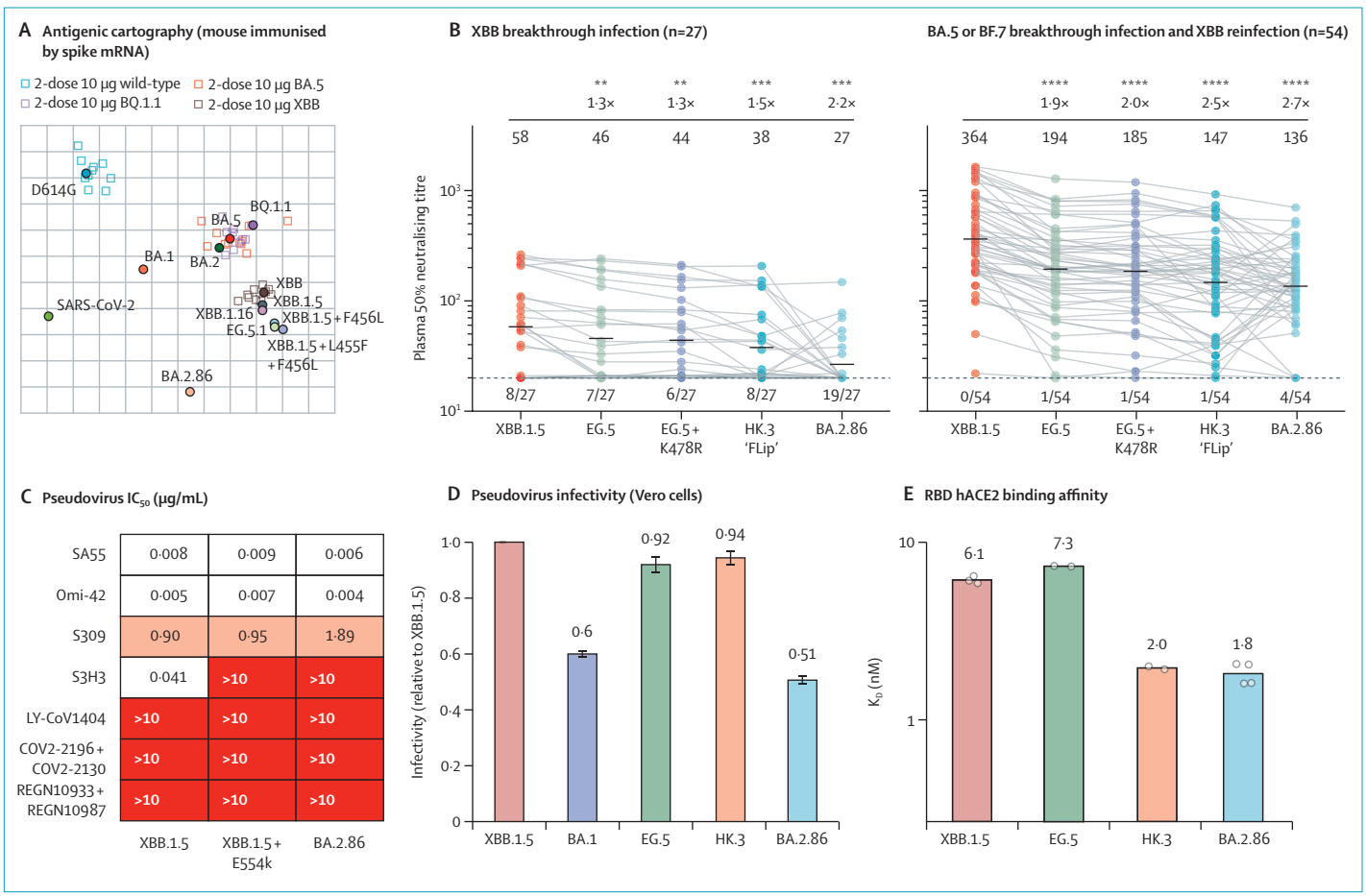

Figure: **Antigenicity, neutralisation resistance, and infectivity of SARS-CoV-2 BA.2.86**
(A) Antigenic cartography on the pseudovirus neutralisation titres of mRNA-immunised mice's plasma against various SARS-CoV-2 strains. Antigens are denoted as coloured circles whereas plasma are shown as squares with the outlines coloured by the corresponding antigens. The distances between plasma and an antigen are negatively correlated to the neutralisation ability. (B) The 50% neutralising titre against SARS-CoV-2 XBB subvariants and BA.2.86 of convalescent plasma from individuals who received triple doses of CoronaVac and breakthrough infections of XBB subvariants with S486P substitution (n=27), or BA.5 or BF.7 breakthrough infections followed by XBB subvariants plus 486P reinfection (n=54). Statistical significances and geometric mean titre fold changes are labelled in comparison with neutralisation against XBB.1.5. Geometric mean titres are labeled above each group. Numbers of negative samples are labelled below the dashed line that indicates the limit of detection (50% neutralising titre=20). Two-tailed Wilcoxon signed-rank tests of paired samples were used. *p<0·05, **p<0·01, ***p<0·001, and ****p<0·0001. (C) IC50 (μg/mL) of approved or candidate monoclonal neutralising antibody drugs targeting RBD or SD1 on Spike, against XBB.1.5, XBB.1.5 plus E554K, and BA.2.86 pseudovirus. (D) Relative infectivity of BA.1, EG.5, HK.3, and BA.2.86 compared with XBB.1.5. The efficiencies of infecting Vero cells are tested using vesicular stomatitis virus-based pseudoviruses. Error bars indicate the mean ± SD of three replicates. The p values are as follows: for BA.1, p=0·0016; for EG.5, p=0·16; for HK.3, p=0·26; and for BA.2.86, p=0·0043. Mean values are labelled above each bar. Two-tailed Student's t-tests were used to calculate the p values. (E) Human ACE2-binding affinity of XBB.1.5, EG.5 (XBB.1·5 plus F456L), HK.3 (EG.5 plus L455F), and BA.2.86 RBD established by surface plasmon resonance. All replicates are shown as points. Geometric mean K_D values (nM) are shown above the bars. ACE2=angiotensin-converting enzyme 2. IC_{50}=Half-maximal inhibitory concentration. RBD=receptor binding domain.

cellular infectivity by testing the efficiency of its pseudovirus form to infect Vero cells and human ACE2-HEK293T cells (figure D; appendix 1 p 10). Among all tested strains, BA.2.86 had the lowest infectivity compared with XBB.1.5, EG.5, and HK.3. To figure out if the compromised infectivity could be attributed to ACE2-binding affinity, we constructed and expressed BA.2.86 recombinant RBD and tested their affinity to human ACE2 by surface plasmon resonance. BA.2.86 had higher human ACE2 binding than XBB.1.5 and EG.5, indicating that the low infectivity in vitro should be attributed to other factors, probably the altered dynamics of RBD up and down transition or the efficiency of membrane fusion (figure E; appendix 1 p 10). Of note, the infectivity measured here is obtained through pseudovirus assays, which should be confirmed by assays using authentic BA.2.86 isolates. Additionally, the efficiency of infecting cell lines tested in vitro could not be directly related to real-world transmissibility, which is much more complex and should be revealed by careful epidemiological tracing.

In summary, we found that BA.2.86 is antigenically distinct from XBB.1.5 and previous Omicron variants, and can evade XBB-induced and XBB-effective neutralising antibodies targeting various epitopes. Therefore, the efficacy of developing XBB-based vaccines against BA.2.86 should be closely monitored and carefully

evaluated. Similar to early results from another saltation variant, Omicron BA.1, and BA.2.86 has a lower efficiency in infecting cell lines in vitro.[9] However, BA.2.86 might obtain additional mutations during its transmission to enhance its infectivity, similar to the previous convergent evolution of S486P in XBB subvariants, which highlights the necessity of global cooperation to track the evolution of BA.2.86.[10]

YC applied for a provisional patent for BD series antibodies, which includes BD55–5514 (SA55), and is the founder of Singlomics Biopharmaceuticals. All other authors declare no competing interests.

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