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Letter

SARS-CoV-2 breakthrough infections following inactivated vaccine vaccination induce few neutralizing antibodies against the currently emerging Omicron XBB variants

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Dear Editor,

COVID-19 inactivated vaccines have been extensively administered in China. However, the majority of the Chinese population has experienced breakthrough infections from SARS-CoV-2 ancestral, Delta or Omicron variants over the past three years, particularly during the wave of Omicron BA.5 and BA.7 variants at the end of 2022 (Zhu et al., 2023). Subsequently, new Omicron variants, such as BQ.1, BQ.1.1, XBB, XBB.1/XBB.1.9, and XBB.1.5/XBB.1.9.1, are emerging in China (Yue et al., 2023; Zhu et al., 2023). Therefore, it is an urgent need and a public health imperative to assess the extent of the immunoprotection established in this population.

In a previous study, using VSV-based pseudovirus system, we observed significant neutralization escape by Omicron BA.2, BA.3, and BA.4/5 variants among individuals who had received several doses of inactivated vaccines and subsequently infected with ancestral, Delta, or Omicron BA.1 variant. This raises concerns regarding the potential for reinfection, particularly with emerging Omicron sublineages BA.4/5 variants, in this population (Shen et al., 2023). The subsequent course of the pandemic in China aligns with these findings, supporting both feasibility and reliability of our assessment method.

In this study, to investigate the neutralization escape of newly emerging variants in individuals previously infected with SARS-CoV-2 who had received inactivated vaccines, we included 91 serum samples obtained from SARS-CoV-2 convalescent individuals during the ancestral, Delta, Omicron BA.1 and BA.5 waves in Yunnan Province between June 2021 and January 2023. All the samples were collected one week after recovery. The participants received none, two, or three doses of the homologous and heterologous inactivated vaccines BBIBP-CorV and CoronaVac, and had experienced infection with one of the SARS-CoV-2 variants only once. Their relevant information was summarized in Supplementary Table S1. Using the VSV-based pseudovirus assays, we systematically assessed the neutralizing activity of these sera against the current BF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 variants.

Briefly, during the initial surge of the ancestral SARS-CoV-2 in Yunnan Province in June 2021, we obtained 22 convalescent serum samples. Of them, 7 individuals had not received inactivated vaccine, and 15 had received two doses of inactivated vaccine prior to infection. In the unvaccinated group, the geometric mean neutralization titers (GMTs) against BA.5 and BF.7 exhibited similar fluctuations at a low level, measuring 34 and 33, respectively. However, GMTs for BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 were all less than 10, with nearly no nAbs detected against XBB.1.5 in all individuals (Fig. 1A). In the vaccinated group, the titers against Omicron BA.5 and BF.7 increased by 6.5- and 7.9-fold, respectively, compared to the unvaccinated group (Fig. 1A and B). Nevertheless, breakthrough infection with ancestral strain still failed to induce higher levels of nAbs against BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5, with extremely low GMTs of 29, 25, 11, <10, and <10, respectively (Fig. 1B and Supplementary Fig. S1A).

As the Delta variant emerged in Yunnan Province between July 2021 and September 2021, we collected 17 convalescent serum samples, of which 6 had not received inactivated vaccine, and 11 had received two doses of inactivated vaccine before infection. In the unvaccinated group, the nAb titers against BA.5, BF.7, BQ.1, and BQ.1.1 were slightly higher than those in the ancestral strain infection, but still remained relatively

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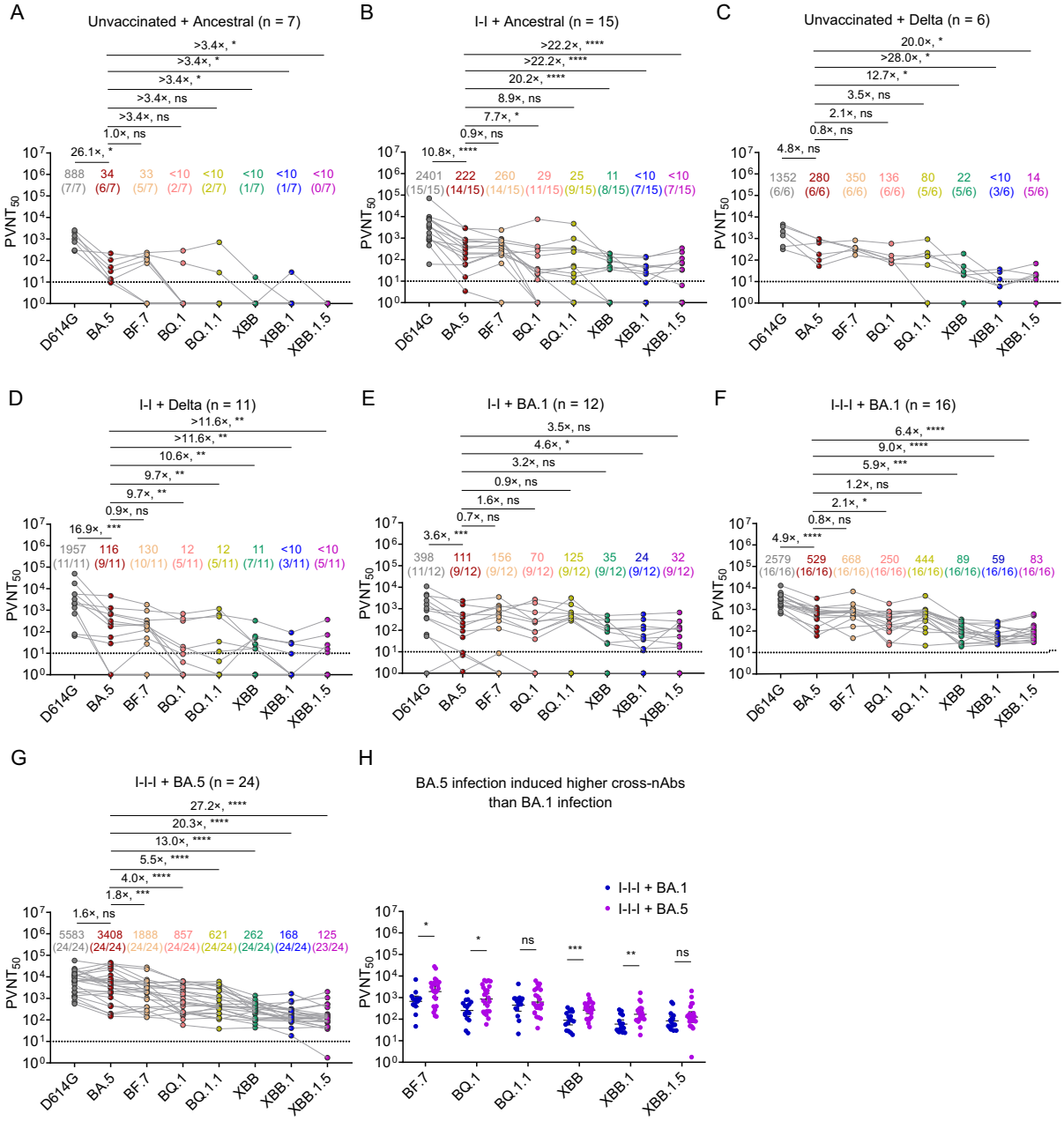

Fig. 1. Serum neutralizing antibodies in individuals induced from previous SARS-CoV-2 infections were assessed to neutralize the current Omicron variants. After the SARS-CoV-2 ancestral (**A** and **B**), Delta (**C** and **D**), Omicron BA.1 (**E** and **F**), or Omicron BA.5 (**G**) variant infection, the convalescent serum samples were collected and cross-neutralizing antibodies (nAbs) against the Omicron BF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 were assessed by VSV-based pseudovirus assays, and the cross-nAbs in Omicron BA.1 and BA.5 were compared to each other (**H**). "I" represented a dose of inactivated vaccine. Each data point represented the $PVNT_{50}$ value from a serum sample. The geometric mean titers (GMTs) for the 50% pseudovirus neutralization titer ($PVNT_{50}$) are shown at the top of the plots. The limit of detection was set as 10 and the proportion of persons' number with $PVNT_{50}$ values above the limit of detection of the total participants was also showed. In (**H**), Data were presented as geometric mean with 95% confidence interval. Statistical comparisons were performed using the two-tailed Wilcoxon matched-pairs signed-rank test (**A**–**G**) and Mann-Whitney *U* test (**H**) (ns, not significant, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$). Decreased fold of GMTs and significant labels were included in all figures.

low at 280, 350, 136, and 80, respectively. The nAb titers against BQ.1 and BQ.1.1 were 2.1- and 3.5-fold lower than that of BA.5. Meanwhile, the nAb titers against XBB, XBB.1, and XBB.1.5 were undetectable (Fig. 1C). In the vaccinated group, breakthrough infection with Delta variant also failed to evoke high levels of nAbs against BF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5, with nearly undetectable GMTs of 130, 12, 12, 11, <10, and <10, respectively (Fig. 1D). No significant elevation was observed when compared to the unvaccinated group (Fig. 1C and Supplementary Figure S1B). This may be due to the robust immune response induced by Delta variant infection, thus weakening the role of vaccine-induced protection (Shen et al., 2023). Together, the low titers of nAbs against BF.7, BQ.1 and BQ.1.1, and the nearly undetectable nAbs against XBB, XBB.1 observed in Delta infection were consistent with those observed in ancestral strain infection (Fig. 1A and B). These results suggest that neither ancestral strain nor Delta variant infection is capable of inducing sufficient cross-nAbs against the circulating Omicron BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 variants, even in individuals who have received two doses of inactivated vaccine.

When the initial Omicron BA.1 wave emerged in Yunnan Province from April 2022 to May 2022, we enrolled 28 convalescent serum

samples. Twelve of them had received two doses of the inactivated vaccine and the remaining 16 had received three doses prior to infection. In the two-dose vaccine group, the titers of nAbs against BA.5, BF.7, BQ.1, BQ.1.1, XBB, XBB.1, and XBB.1.5 were similarly low, with GMTs of 111, 156, 70, 125, 35, 24 and 32, respectively (Fig. 1E). These titers did not show an obviously elevated tendency when compared to counterparts in ancestral strain and Delta variant infections (Fig. 1B and D). In the third-dose vaccine group, slightly higher but no statistically significant nAbs were induced against all the tested variants compared to those in the two-dose vaccine group. Although the GMTs of the nAbs against BA.5, BF.7, BQ.1, and BQ.1.1 rose to moderate levels of 529, 668, 250, and 444, respectively, the nAbs against XBB, XBB.1, and XBB.1.5 were still low, with GMTs of 89, 59, and 83, respectively (Fig. 1F and Supplementary Figure S1C). Thus, these results suggest that breakthrough infections with BA.1 variant does not induce sufficient cross-nAbs against the XBB, XBB.1, and XBB.1.5 variants, even in individuals who have received two or three doses of the inactivated vaccine.

Since the explosive outbreak of Omicron BA.5 variant in Yunnan Province between December 2022 and January 2023, we enrolled 24 convalescent serum samples. All participants had received three doses of the inactivated vaccine before infection. In this group, the GMTs against D614G and BA.5 were remarkably high (5583 and 3408, respectively), indicating that BA.5 infection induced high titers of nAbs against the ancestral strain and itself. Furthermore, moderate levels of nAbs against BF.7, BQ.1, and BQ.1.1 were evoked, with GMTs of 1888, 857, and 621, respectively. These values were reduced by 1.8, 4.0, and 5.5-fold when compared to that against BA.5. Unfortunately, BA.5 infection consistently failed to induce enough cross-nAbs against XBB, XBB.1, and XBB.1.5, with low GMTs of 262, 168, and 125, respectively (Fig. 1G). These results indicate individuals who have recently experienced a BA.5 breakthrough infection remain susceptible to the XBB/XBB.1/XBB.1.5 variants, even after receiving a three-dose inactivated vaccine regimen. However, compared with the BA.1 infection, the BA.5 infection elicited relatively higher levels of cross-nAbs against all the examined variants (Fig. 1H). This finding may be attributed to the higher antigenic similarity of the spikes between BA.5 and XBB/XBB.1/XBB.1.5 sublineages compared to BA.1, as both BA.5 and XBB sublineages are derived from the BA.2 descendant sublineages. Relative to BA.5, BA.1 exhibits 6 sites (F317L, A376T, N405D, S408R, Q493R, and G496S) within the receptor-binding domain (RBD) of its spike that distinguish it from XBB sublineages (Ma et al., 2023).

In our previous study, we found that a strong correlation between increased doses of inactivated vaccine and enhanced protection against the BA.4/5 infection (Shen et al., 2023). However, our present findings disappointingly demonstrate that regardless of whether it is an ancestral (Supplementary Figure S1A), Delta (Supplementary Figure S1B), or BA.1 (Supplementary Figure S1C) breakthrough infection, the increase in inactivated vaccine doses is not related to a more effective neutralization against all the newly emerging variants. Furthermore, when comparing each nAb titer against the newly emerging variant with those against BA.5, we observed varying degrees of neutralizing resistance among these variants as follows: XBB.1.5 $\approx$ XBB.1 $\approx$ XBB $>$ BQ.1.1 $\approx$ BQ.1 $>$ BF.7 $\approx$ BA.5 (Supplementary Figure S1D). This further demonstrates the outstanding neutralization escape of XBB sublineages in the background of inactivated vaccines.

Compared to the BA.5 variant, the enhanced neutralization resistance in XBB/XBB.1/XBB.1.5 may be attributed to a series of unique mutations found in the RBDs, including D339H, R346T, L368I, V445P, G446S, R452L, N460K, V486S, and F490S. Moreover, the additional F486P mutation in XBB.1.5 confers increased receptor-binding capacity and transmissibility (Hoffmann et al., 2023; Yue et al., 2023). Currently, several variants evolved from XBB, including XBB.1.16 (XBB.1 + E180V, K478R, and F486P), XBB1.9.2 (XBB.1 + F486P and Q613H), XBB.2.3 (XBB + D253G, F486P, and P521S), EG.5 (XBB.1.9.2 + F456L), and EG.5.1 (XBB.1.9.2 + Q52H and F456L), have rapidly disseminated across multiple countries (Zhang et al., 2023). These emerging XBB sublineages exhibit comparable or slightly enhanced immune evasion capabilities when compared to the early XBB sublineages (Kaku et al., 2023; Wang et al., 2023; Zhang et al., 2023). However, in the context of inactivated vaccine administration, further investigation is required to determine whether prior infection with early XBBs variants (XBB/XBB.1/XBB.1.5) can provide sufficient protection against these new XBBs.

It is worth noting that significant neutralization escape of XBB, XBB.1, and particularly XBB.1.5 has also been observed in other populations that have received several doses of mRNA vaccines with a BA.5 breakthrough infection (Arunachalam et al., 2023; Yue et al., 2023). Furthermore, other studies suggested that a heterologous booster with an intranasal vaccine or a bivalent vaccine may outperform homologous vaccination strategies, eliciting slightly higher levels of cross-nAbs against some of these newly Omicron variants, including BF.7, BQ.1, BQ.1.1. However, the cross-nAbs against XBB, XBB.1, and XBB.1.5 remained persistently low, even after a BA.2 or BA.5 breakthrough infection (Devasundaram et al., 2023; Hoffmann et al., 2023; Zhu et al., 2023). Our results align with these reports and support the conclusion that the XBB, XBB.1, and XBB.1.5 variants exhibit superior neutralization escape when compared to the well-known BA.5 variant.

Importantly and encouragingly, we observed that a BA.5 breakthrough infection could elicit significantly enhanced cross-nAbs responses against XBB, XBB.1, and XBB.1.5 compared to the previous BA.1 variant. This finding suggests a potential attenuation of virulence in the newly emerging variants relative to their predecessors, indicating a slightly enhanced immuno-barrier for population protection. We should point out that the authentic virus assays were lacked in this study; however, previous reports have indicated concordance between pseudovirus and authentic virus assays in assessing nAb responses (Schmidt et al., 2020).

In summary, this study presents a systematic description of the intricate cross-nAb generation in the individuals who received an inactivated vaccine and subsequently encountered ancestral, Delta, Omicron BA.1, or BA.5 variant infection. Our findings demonstrate the remarkable immune evasion capabilities of XBB, XBB.1, and XBB.1.5 variants in these individuals, emphasizing the importance of personal protection and the urgent need for the development of broad-spectrum COVID-19 vaccines for this particular population.

Footnotes

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