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LETTER TO THE EDITOR

Repeated vaccination of inactivated SARS-CoV-2 vaccine dampens neutralizing antibodies against Omicron variants in breakthrough infection

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Dear Editor,

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019, the virus has continued to evolve resulting in new waves of infection and immune escape in the vaccinated population.[1] The Omicron strains belong to newly prevailing variants of concern (VOCs). They acquired as many as 30 mutations in the spike (S) gene and half of which occur at the receptor-binding domain (RBD) of the S protein. Moreover, the Omicron variants can evade neutralization activity by most of the identified anti-SARS-CoV-2 antibodies.[2,3] Several reports have suggested that three-dose vaccination mounted better neutralizing activity against Omicron than two-dose vaccination.[4] However, it is not clear how breakthrough infection would affect the immune responses of those who had three-dose compared to two-dose vaccination. Here, we compared neutralizing antibody (nAb) levels according to a vesicular stomatitis virus (VSV) pseudovirus-based neutralization assay in people who experienced breakthrough infection after receiving either two or three doses of inactivated SARS-CoV-2 vaccine during the Omicron BA.2 wave in Shanghai between March and June 2022. Strikingly, we found that although nAb titers against SARS-CoV-2 were comparable between the 2-dose and the 3-dose groups of patients with BA.2 breakthrough infection, nAb titers against the Omicron BA.2, BA.4 and BA.5 variants were significantly lower in the 3-dose group. Our data suggest that repeated vaccination with inactivated virus vaccine back-boosts previous memory and dampens the immune response to a new antigenically related but distinct viral strain. Such vaccination-induced immune imprint could reflect the "original antigenic sin" doctrine described in the influenza field, whereby individuals infected with a new circulating viral strain developed a strong immune response to a priorly exposed strain.[5] Thus, careful considerations in this aspect should be taken when designing future vaccination and booster strategies.

Since the rollout of vaccine based on the original SARS-CoV-2 strain in early 2021, ~91% of the population in China had received a full primary schedule and 53% of the vaccinated population had received a booster dose.[6,7] Despite the high vaccination rate, 0.63 million people were infected during the Omicron BA.2 wave in Shanghai between March and June 2022. The BA.2 variant acquired 29 mutations in the viral S protein including 16 mutations in the RBD region (Supplementary information, Fig. S1a), resulting in the escape from vaccine-induced nAbs. In this study, we tested how vaccination strategies with two or three doses of inactivated virus vaccine followed by infection with BA.2 contributed to nAb activities against the original SARS-CoV-2, the infected strain BA.2, and other circulating variants including BA.4 and BA.5.

Out of the 135 serum samples collected from BA.2-infected patients in Shanghai, 24 people had never received any SARS-CoV-2 vaccination, 56 people had received two doses of inactivated SARS-CoV-2 vaccine, while the remaining 55 people had received three doses of inactivated SARS-CoV-2 vaccine prior to BA.2 infection or breakthrough infection (Fig. 1a). This study cohort consisted of 64 male and 71 female patients with ages of 22–96 years old (84% of the patients were > 50 years old) (Supplementary information, Fig. S1b and Table S1). Serum samples were collected within 38 days of SARS-CoV-2 virion-positive test date, where the average was 9 days for both the 2-dose and 3-dose vaccinated groups (Supplementary information, Table S2). 62 of the patients had pre-existing comorbidities, amongst whom 25 had received 2 doses of vaccine and 24 had received 3 doses of vaccine (Supplementary information, Table S3). While the unvaccinated group presented several severe clinical symptoms, the 2-dose and 3-dose groups mostly presented mild symptoms (Fig. 1b; Supplementary information, Table S4).

To test the immune responses during Omicron infection in patients with different vaccination backgrounds, we measured nAb titers in the serum samples against the original SARS-CoV-2 strain and the Omicron variants including BA.2, BA.4 and BA.5 using a VSV pseudovirus-based neutralization assay[8] (See Supplementary information, Data S1). The 50% pseudovirus neutralizing titers ($pVNT_{50}$) of each sample in the SARS-CoV-2 and the Omicron variant pseudovirus assays were determined. The percentages of samples with undetectable neutralizing activities ($pVNT_{50} < 45$) decrease with increased vaccination dose, 62.6% for the unvaccinated group (15 out of 24), 44.6% for the 2-dose group (25 out of 56) and 36.4% for the 3-dose group (20 out of 55) (Fig. 1c; Supplementary information, Tables S5 and S6). However, the proportions of people with undetectable or detectable nAbs were not significantly different between the 2-dose and the 3-dose groups (Supplementary information, Fig. S1c). Notably, although a higher percentage of people who received three doses of vaccine had high anti-SARS-CoV-2 nAb titers ($pVNT_{50} > 1000$) compared to those who received two doses of vaccine, significantly lower percentages of people who received three doses of vaccine exhibited high anti-Omicron nAb titers ($P = 0.0103$ for BA.2 and $P = 0.0008$ for BA.4) (Fig. 1c; Supplementary information, Fig. S1c). This result suggests that the antibodies that were generated by repeated vaccination (3-dose) had greater neutralizing activities towards the vaccinated strain than the newly infected strain or other circulating strains.

Next, we plotted the nAb titers of the samples that were above the detection threshold ($pVNT_{50} > 45$) and calculated the geometric mean titers (GMTs) of each vaccine dose group. The GMTs of nAbs against the original SARS-CoV-2 was the highest in the

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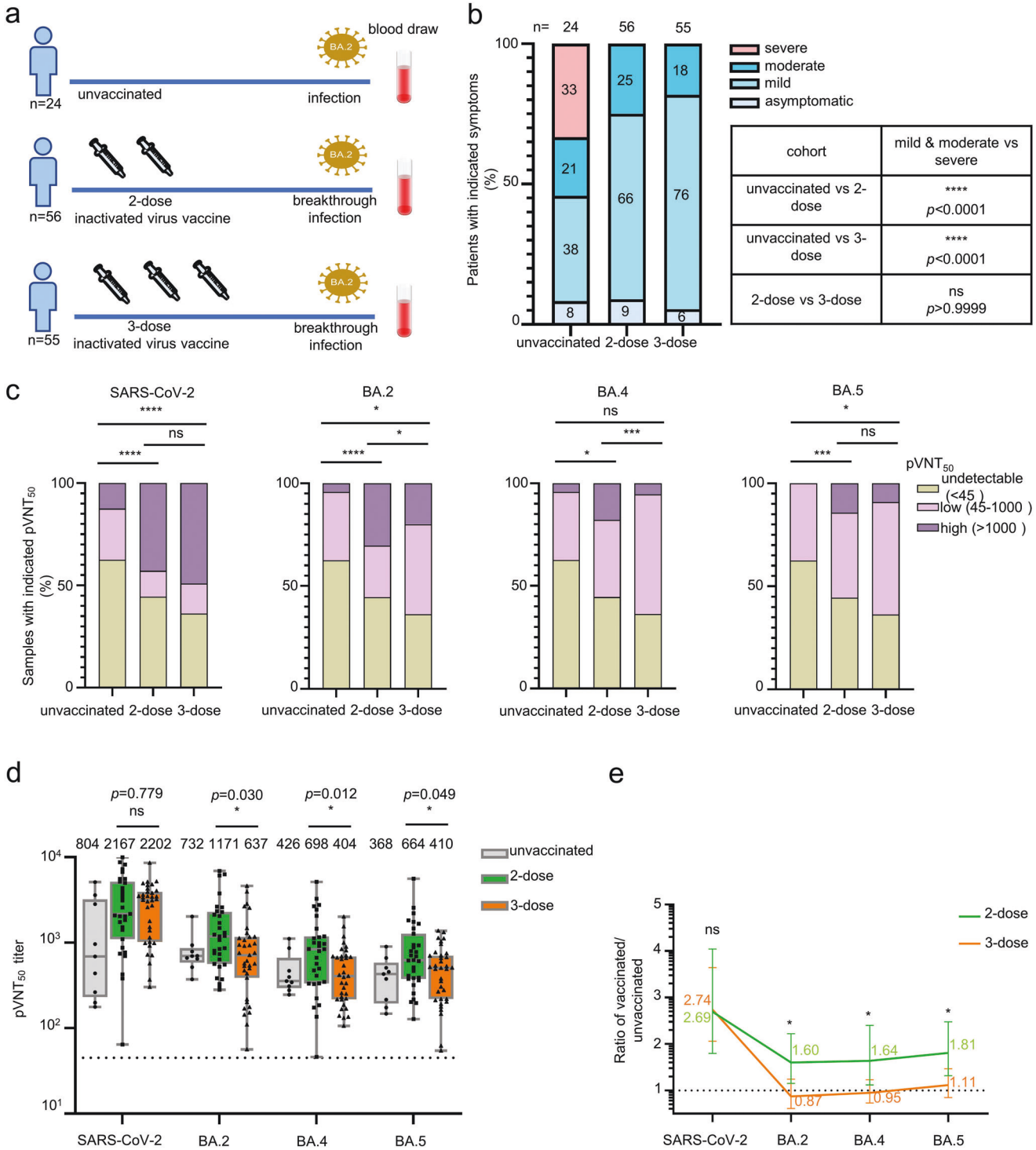

Fig. 1 Comparisons of pseudovirus neutralizing titers in BA.2-infected individuals with different vaccination backgrounds. a The schematic diagram shows the number of unvaccinated, 2-dose and 3-dose vaccinated individuals prior to BA.2 infection or breakthrough infection. **b** The stacked bars represent the proportion of all BA.2-infected patients, presenting with the indicated clinical symptoms. The numbers shown in the bar and above the bar represent the percentages of people with the indicated clinical symptoms and the total number of people in each vaccination group, respectively. Two-tailed χ^2 tests were performed in **b**. ****$P < 0.0001$; ns, not significant. **c** The percentages of individuals with the indicated $pVNT_{50}$ values in SARS-CoV-2, BA.2, BA.4 and BA.5 pseudovirus neutralization assays are displayed. **d** Box plots show the $pVNT_{50}$ values of samples that are above the detection limit. Each black dot represents a sample. The whiskers show the minimum and maximum $pVNT_{50}$ titers. The line in the box represents median. The GMTs of each group are indicated in numbers on the top of each box plot. Black dotted line represents the detection threshold. **e** The ratio of the GMTs of the 2-dose or 3-dose group over the unvaccinated group is shown. Orange numbers indicate the ratio of the GMT of the 3-dose group over the unvaccinated group. Green numbers indicate the ratio of the GMT of the 2-dose group over the unvaccinated group. Statistical analysis was performed using unpaired two-tailed Mann-Whitney tests. *$P < 0.05$; ns, not significant.

group that received three doses of vaccine. However, it was not significantly different from the GMT of the 2-dose group (Fig. 1d). Strikingly, the group that received three doses of vaccine had significantly lower GMTs of nAbs against BA.2, BA.4 and BA.5 when compared to the group that received only two doses of vaccine (Fig. 1d, e). To exclude the possibility that the bias observed was due to age discrepancy, we performed the same analysis on samples that were below 80 years old, and observed a similar trend (Supplementary information, Fig. S1d). nAb titers could be correlated to clinical symptoms and disease severity.[9] To exclude the possibility that the lower titer of nAbs against Omicron variants was due to more mild symptoms in the 3-dose group, we analyzed the clinical symptoms of patients with detectable nAbs. The percentages of patients with mild symptoms were the same between the 2-dose and the 3-dose groups (68%), suggesting that the lower anti-BA.2 nAb titers in the 3-dose group were not because this group presented more mild symptoms (Supplementary information, Fig. S2a, b). Furthermore, by plotting the nAb titers of SARS-CoV-2 and BA.2, we found that ~22.9% of the samples in the 3-dose group exhibited low BA.2-specific and medium/high SARS-CoV-2-specific nAb titers, but none in the 2-dose group with the same criteria (Supplementary information, Fig. S2c–h). Notably, in an anti-SARS-CoV-2 RBD antibody depletion assay in selected serum samples with high levels of SARS-CoV-2 nAb titers, we found that a significantly greater percentage presented a dramatic decline of anti-SARS-CoV-2 nAb titers in the 3-dose group as compared to the 2-dose group (68.4% vs 33.3%, $P < 0.0001$), indicating that antibodies targeting the RBD of the original strain made a larger contribution in serum neutralization of the 3-dose group (Supplementary information, Fig. S3a–c). Collectively, these results suggest that repeated vaccination of inactivated SARS-CoV-2 vaccine dampens the nAb response against the new Omicron variants in breakthrough infection due to stronger immune imprint on the ancestral strain.

The rapid development of vaccine against SARS-CoV-2 in the early pandemic greatly reduced the mortality and severity of COVID-19 cases.[10] However, the emergence of VOCs that evade immune response has continued to pose threats to human health. Future vaccination and booster strategy to battle with new variants would have to consider the impact of existing immune memory since a majority of the population has now gained some sort of immunity either through vaccination or natural infection with the original SARS-CoV-2 or variants and breakthrough infection with new variants. Our data suggest that repeated vaccination with inactivated virus vaccine may recall strong immune response to target the original strain, which in turn dampen immune responses to newly infected strains, as manifested by reduced nAb activities against BA.2, BA.4 and BA.5 in the group that received three doses of vaccines. This data was in line with observations by others that breakthrough infection resulted in antibody responses against the original strain and recalled memory B cells that target the RBD of the original strain.[11,12] Although we found that the nAb activities against the Omicron variants were lower in the 3-dose group compared to the 2-dose group, the clinical symptoms were comparable between these two groups, and three doses of vaccine have been proven to be useful against severe symptoms and deaths. The conclusion of this work is limited to patients with breakthrough infection, and for most people, a booster dose is still highly recommended for protection against severe COVID-19. Future studies investigating how population variations affect nAb titers and immune responses to infection and vaccination would be critical. Our results call for caution on the immune imprint when designing future vaccination and booster strategies. A possible solution to such immune imprint, as put forward by the influenza field, is the design of an universal vaccine that can elicit broad nAbs against current and future related strains.[5] For the vaccinated population, vaccines targeting the conserved fusion peptide at the S protein subunit 2 (S2),[13–15] would seem promising as an alternative choice for sequential vaccination and would be an important line of future investigation.

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AUTHOR CONTRIBUTIONS

Y.W., L.-S.Y. and B.G. designed the experiments and wrote the manuscript. B.G. and L.H. performed pseudovirus neutralization assay. J.X., Y.B. and G.L. collected the clinical samples. Y.C., L.H. and Y.B. collected the clinical data. Y.Z. and Y.X. provided reagents and cell lines. B.S. edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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