INTRODUCTION

The recent emergence of the SARS-COV-2 variant Omicron (B.1.1.529) has become a global concern, causing a super-spreader event that appears to be at least as infectious as Delta (B.1.617.2) and has surpassed Delta in epidemic trends in several countries and regions within weeks. The detection of mutations in the RBD region of the spike protein raises concerns that vaccine immunity will be
compromised. Compared with other mutants, the Omicron variant contains more than 15 changes in RBD domain of the spike protein, which is the main target of vaccine-induced immunity. Based on the number and location of replacements, as well as on data from other variants with similar spike protein replacements, a significant reduction in serum neutralization activity is expected in vaccinated or previously infected individuals, which may indicate reduced protection against infection. A recent study showed that the secondary breakthrough infection rate of household confirmed Omicron was about 21.6%, which is twice that of the Delta variant.

In clinical trials, SARS-CoV-2 vaccines have shown absolute health benefits by inducing neutralizing humoral and cellular immunity and reducing the number of COVID-19 infections, hospitalizations, and deaths. However, it has now been shown that neutralizing antibody responses and vaccine potency vary from vaccine dose to vaccine dose, decrease over time after vaccination, and are negatively affected by emerging mutations. The situation is further exacerbated by the observation that neutralizing antibody levels and vaccine protection decreased six months after vaccination, and it has been agreed that protection against emerging variants should be enhanced by increasing doses. However, while vaccines against early variants have been shown to effectively neutralize other variants, it is not clear whether this correlation will be maintained in immune-boosting and highly mutated variants like Omicron.

To answer these questions, we measured the neutralizing activity in vitro for Omicron and compared it with wild type (WH-09) and Delta variants in sera from humans and monkeys with different levels of immunity. The monkey sera samples were collected at 1 and 3 months post three-dose inactivated (PiCoVacc) and recombinant protein (Anhui Zhifei Longcom Biopharmaceutical) vaccination, and human sera samples were collected at 1 month post three-dose inactivated (PiCoVacc) vaccination.

2 | METHODS

2.1 | Cells and viruses

The SARS-CoV-2 viruses designated as SARS-CoV-2/human/CHN/Delta-1/2021 (Genbank: OM016195), SARS-CoV-2/human/CHN/Omicron-1/2021 (Genbank: OM095411) and SARS-CoV-2/human/CHN/WH-09/2020 (Genbank: MT093631) were provided by ILAS, PUMC, China. Vero E6 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin, and incubated at 37°C, 5% CO₂. Titers for SARS-CoV-2 were resolved by a 50% tissue-culture infectious dose (TCID₅₀) assay.

2.2 | Viral titer

The virus droplets were 10-fold diluted and inoculated into simple VeroE6 cells, incubated at 37°C. One hour later, the dilute solution was added to 200 μL DMEM medium with 2% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated at 37°C, 5% CO₂. Three days later, we observed cytopathic effects. The Reed-Muench method was used to calculate tissue culture infectious dose (TCID₅₀).

2.3 | Neutralizing antibody assay

We used cytopathic effect (CPE) tests to detect the presence of neutralizing antibodies. Briefly, human or monkey sera samples were heat inactivated at 56°C. Thirty minutes later, we diluted the sera samples 2-fold serially and then incubated them with 100 TCID₅₀ SARS-CoV-2 at 37°C, One hour later the samples were added to Vero-E6 cells in a 96-well-plate, and cultured at 37°C. After 3–4 days, we observed cytopathic effects, and the sera dilution in which 50% of the cells were protected against infection was calculated.

2.4 | Immunization program

Rhesus macaques (4–7 years old) were divided into two groups. One group was injected intramuscularly with a 3 μg dose of inactivated vaccine three times, on days 0, 29 and 155 (n = 8, PiCoVacc). The second group was injected intramuscularly with a 25 μg dose of recombinant protein vaccine at the same time points (n = 8, ZF2001, Anhui Zhifei Longcom Biopharmaceutical). Blood samples were collected at 1 and 3 months post three-dose vaccination to analyze the neutralizing titers.

Human blood samples were collected from 6 volunteers in our laboratory at 1 month post three-dose inactivated vaccine, with the second dose injected 6 months later.

2.5 | Statistical analysis

We use GraphPad Prism 8.0 software (GraphPad Software, Inc) to analyze all data, using a two-tailed unpaired Student’s t test for comparisons among groups. The levels of statistical significance were determined as p < 0.05 (*), p < 0.01 (**).

3 | RESULTS

3.1 | Cross-neutralizing antibodies elicited by inactivated vaccine boosters in monkey sera

We measured the neutralizing activity in vitro for Omicron and Delta variants compared with wild type (WH-09) in monkey sera samples collected at 1 and 3 months post three-dose inactivated vaccine. GMTs of NAb for sera against wild type (WH-09), Delta, and Omicron variants were found to be 210.6/74.7, 324.8/61.3,
and 43.3/14.3, respectively. GMTs of NAb against the Omicron variant was 4.9/5.2-fold lower than those of the wild type virus (Figure 1).

3.2 | Cross-neutralizing antibodies elicited by recombinant protein vaccine boosters in monkey sera

We measured the neutralizing activity in vitro for Omicron compared with wild type (WH-09) and Delta variants in monkey sera samples collected at 1 and 3 months post three-dose recombinant protein vaccination, GMTs of NAb for sera against wild type (WH-09), Delta and Omicron variants were found to be 210.5/69.9, 219.9/52.6, and 13.4/7.9, respectively. GMTs of NAb against the Omicron variant was 15.7/8.9-fold lower than those of the wild type virus (Figure 2).

3.3 | Cross-neutralizing antibodies elicited by inactivated vaccine boosters in human sera

We measured the neutralizing activity in vitro for Omicron compared with wild type (WH-09) in human sera samples collected at 1 month post three-dose inactivated vaccination. GMTs of NAb for sera against wild type (WH-09) and Delta were found to be 34.0 and 10.8, respectively. GMTs of NAb against the Omicron variant was 3.1-fold lower than that of the wild type virus (Figure 3).

4 | DISCUSSION

In the absence of Omicron mutation-specific vaccines, approved vaccines remain the strategy to reduce severe illness and high mortality caused by current circulating strains, including...
Omicron. This study is a preliminary assessment of the neutralization for Omicron variant using vaccine sera to explore whether the Omicron variant can escape the immunity barrier previously established by vaccine immunity. Because the response of the neutralizing antibody and the efficacy of the vaccine vary with the vaccine agent, and high neutralizing antibody titers have been confirmed in individuals vaccinated with boosters, we used two different types of vaccines, inactivated and recombinant protein vaccines, for booster immunization, and different collection times post vaccination to assessed the neutralization for the Omicron variant.

The neutralizing antibody (NAb) titers of inactivated and recombinant protein vaccine against the Omicron variant were, respectively, 4.9/5.2-fold and 8.9/15.7-fold lower than wild type (WH-09) in monkey sera, and a similar trend was observed in the human sera, where the NAb titers of the Omicron variant were 3.1-fold lower than the wild virus. We also found that the neutralizing antibody responses decreased with time post vaccination. Our results are consistent with previous studies. Fifteen mutations were observed in the RBD domain of Omicron, of which three mutations (K417, E484, N501) were similar in the Gamma and Beta variants. Prior work demonstrated that the Omicron variant can escape antibodies and is more resistant to neutralization by vaccine sera. Our study showed that despite a reduction in neutralization titers against Omicron variants of sera containing inactivated and protein vaccines, their neutralization potential is well established.

Compared to inactivated vaccine, recombinant protein vaccine had a lower neutralizing activity, which might be related to the extensive epitope coverage in inactivated vaccines, which induces an immune response against the entire virus particle, thereby reducing the vaccine’s ability to neutralize newly emerging mutations.

The limitations of our study included the relatively small sample sets and limited longitudinal data, so further larger-scale studies need to be carried out to assess the level and durability of protection against Omicron provided by different types of vaccines.
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CONFLICT OF INTEREST
The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS
Qi Lv, Shasha Zhou and Linlin Bao conceived and designed the study; Qi Lv, Shasha Zhou, Yaqing Zhang Fengdi Li and Mingya Liu performed the experiments; Shasha Zhou and Feifei Qi collected and analyzed the data; Qi Lv wrote the original draft of the manuscript; Linlin Bao revised the manuscript. All authors critically read and contributed to the manuscript and approved the final version.