1 **Impact of various vaccine boosters on neutralization against Omicron following**

2 **prime vaccinations with inactivated or adenovirus-vectored vaccine**

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20

21 **Abstract**

22 Since the first report on November 24, 2021, the Omicron SARS-CoV-2 variant is

23 now overwhelmingly spreading across the world. Two SARS-CoV-2 inactivated

24 vaccines (IAVs), one recombinant protein subunit vaccine (PRV), and one adenovirus-

25 vectored vaccine (AdV) have been widely administrated in many countries including

26 China to pursue herd immunity. Here we investigated cross-neutralizing activities in

27 341 human serum specimens elicited by full-course vaccinations with IAV, PRV and

28 AdV, and by various vaccine boosters following prime IAV and AdV vaccinations. We

29 found that all types of vaccines induced significantly lower neutralizing antibody

30 titers against the Omicron variant than against the prototype strain. For prime

31 vaccinations with IAV and AdV, heterologous boosters with AdV and PRV,

32 respectively, elevated serum Omicron-neutralizing activities to the highest degrees. In

33 a mouse model, we further demonstrated that among a series of variant-derived RBD-

34 encoding mRNA vaccine boosters, it is only the Omicron booster that significantly

35 enhanced Omicron neutralizing antibody titers compared with the prototype booster

36 following a prime immunization with a prototype S-encoding mRNA vaccine

37 candidate. In summary, our systematical investigations of various vaccine boosters

38 inform potential booster administrations in the future to combat the Omicron variant.

Introduction

SARS-CoV-2 variant B.1.1.529, first reported on November 24, 2021, is now rapidly

spreading across the world, especially in regions where the Delta variant is

circulating, suggesting its potential of overtaking Delta to become the next dominant

variant. This variant bears up to 37 mutations in the spike protein including 15 within

the receptor binding domain (RBD) (*1*) , the primary target of SARS-CoV-2

neutralizing antibodies (*2-4*), which raises immense concern on immune evasion. On

November 26, 2021, the World Health Organization (WHO) designated B.1.1.529 as

the fifth variant of concern (VOC) and named it Omicron (*5, 6*). Two SARS-CoV-2

inactivated vaccines (IAVs, CoronaVac by Sinovac and BBIBP-CorV by Sinopharm)

with a two-dose vaccination regimen, one recombinant protein subunit vaccine (PRV,

ZF2001 by Anhui Zhifei Longcom) with a three-dose vaccination regimen, and one

single-dose recombinant adenovirus-vectored vaccine (AdV, Convidecia by CanSino)

have been given conditional approval for general public use or approved for

emergency use by China (*7-11*). These four vaccines form the core of China's

vaccination program. To date, several billion doses of those vaccines have been

widely administered in many countries, including China, with the aim of achieving

herd immunity against SARS-CoV-2. Moreover, to combat wanning vaccine-elicited

immunity with time and emerging variants, several clinical trials with homogenous or

heterogenous platform vaccine boosters have also been conducted (*12-14*).

Recent preliminary studies have reported that neutralization elicited by one mRNA

vaccine (BNT162b2 by BioNTech in collaboration with Pfizer) is substantially

61   reduced against the Omicron variant (*15, 16*). The Omicron variant also escapes the

62   majority of current therapeutic monoclonal antibodies (*17, 18*). It is urgent to assess

63   residual neutralization levels against the Omicron variant that are afforded by widely

64   used vaccines in China, including IAV, PRV, and AdV; it is equally or even more

65   important to investigate which vaccine booster strategy is able to maximize

66   neutralization capacity against the Omicron variant. In this study, we systematically

67   assessed cross-neutralization activity of human antisera against the Omicron variant

68   elicited by infection or full-course vaccinations with IAV, PRV, or AdV, and by

69   homologous or heterogenous vaccine boosters. All of the currently approved SARS-

70   CoV-2 vaccines use the prototype strain-derived proteins as vaccine immunogens. In a

71   mouse model, we further investigated Omicron-neutralizing activity increase in

72   magnitude induced by various booster vaccine candidates that were developed based

73   on different SARS-CoV-2 variants so as to yield key knowledge about guiding

74   potential booster shots in the future.

75   **Results**

76   To investigate the Omicron variant's sensitivity to immunity elicited by infection or

77   full-course vaccination, we measured the binding, blocking and neutralizing activities

78   of serum specimens obtained from 25 convalescent individuals (one month since

79   convalescence), from 30 recipients of two-dose IAV (one month since receipt of the

80   second dose), from 30 recipients of one-dose AdV (one month since receipt of the

81   injection), and from 28 recipients of three-dose PRV (six months since receipt of the

82   third dose) (Table S1). For all of the serum specimens, the binding antibody titers

83 were 5–15 times lower for the Omicron than variant for the prototype strain (Fig. 1A).

84 Notably, except for one specimen from an individual receiving PRV vaccinations

85 scored as positive, all of the other specimens lost the blocking activity against the

86 Omicron variant, whereas nearly all of the specimens exhibited positive blocking

87 activity against the prototype strain (Fig. 1B). Pseudovirus-based neutralization assays

88 demonstrated that the geometric mean NT_{50} titers against the Omicron variant were

89 20-, 10-, 6-, and 4-fold lower than those against the prototype strain in serum

90 specimens from convalescent patients, IAV recipients, AdV recipients, and PRV

91 recipients, respectively (Fig. 1C). In addition, despite being obtained six months

92 following full-course vaccination, the serum samples from individuals who had

93 received PRV vaccinations had the highest neutralizing NT_{50} titers against the

94 Omicron variant, but these titers were approximately 1/5 of the titer of convalescent

95 sera against the prototype strain (Fig. 1C). In contrast, the neutralizing activity against

96 the Omicron variant afforded by full-course vaccination with IAV or AdV was less

97 than 1/20 of the activity of convalescent sera against the prototype strain (Fig. 1C),

98 suggesting a potential of neutralizing insufficiency against Omicron infection.

99 　　To explore the impact of a homologous or heterologous booster at 4–8 months

100 following IAV full-course vaccination on vaccine-induced antibodies against the

101 Omicron variant, we obtained 42 serum specimens from participants receiving no

102 vaccine booster, 39 serum specimens from participants receiving homologous IAV

103 booster (IAV-b) and 45 serum specimens from participants receiving heterologous

104 PRV booster (PRV-b) (Table S1). The samples were from a single-center, open-label,

105   randomized controlled clinical trial at Beijing Ditan Hospital. An additional seven

106   specimens from individuals who had received heterologous AdV vaccine booster

107   following two doses of IAVs 4–8 months earlier from Peking Union Medical

108   University Hospital were also included (Table S1). Binding, blocking and neutralizing

109   antibodies titers in all of the groups were markedly higher against the prototype strain

110   than against the Omicron variant (Fig. 2). The booster dose with IAV, PRV, and AdV

111   vaccine induced 3-, 7-, and 40-fold increase in Omicron-binding antibody titers

112   compared with the no-booster control (Fig. 2A). At 4–8 months after full-course

113   vaccination with IAV, Omicron-blocking antibodies were close to or below the lower

114   limit of detection (4-fold dilution of plasma), and the blocking positive rate was only

115   2% (Fig. 2B and Fig. S1). The IAV, PRV, and AdV vaccine boosters led to an increase

116   of blocking positive rates to 54%, 71%, and 57%, respectively (Fig. 2B and Fig. S1).

117   For neutralizing antibodies against the Omicron variant, the genomic mean NT_{50} titers

118   were below the lower limit of detection (10-fold dilution of plasma) in the control

119   group, whereas the titers rose to 113, 207, and 709 in the IAV, PRV and AdV booster

120   groups, respectively (Fig. 2C and Fig. S2), indicating that the heterologous vaccine

121   booster with AdV was superior to the homologous IAV vaccine booster in improving

122   the neutralizing activity against the Omicron variant.

123   To examine the effect of various booster vaccinations following a single-dose prime

124   vaccination with AdV, we conducted similar tests with serum specimens from

125   individuals receiving no booster injection (control, n=30), IAV booster (n=30), PRV

126   booster (n=30), or AdV booster (n=30) at 4–8 months following the primary AdV

127   vaccination (Table S1). All of the samples were collected one month following

128   booster vaccine injection at Chinese PLA General Hospital. The Omicron-binding

129   antibody titers were boosted 4-, 25- and 16-fold by the booster injection of

130   heterologous IAV and PRV vaccines or homologous AdV vaccine, respectively,

131   compared with the no-booster control (Fig. 3A). For Omicron-blocking antibodies, the

132   PRV and AdV booster groups exhibited an identical blocking positive rate (80%),

133   which was higher than that of the control (3%) or IAV booster group (53%) (Fig. 3B

134   and Fig. S3). The neutralizing NT_{50} titers for the Omicron variant in the control group

135   and the IAV, PRV, and AdV booster groups were 15, 68, 313, and 228, respectively

136   (Fig. 3C and Fig. S4). Notably, a heterologous PRV booster induced the highest

137   degree of neutralizing immunity against both the prototype and the Omicron strains

138   compared with the no-booster control and the other two boosters (Fig. 3C and Fig.

139   S4).

140   All four of the above-mentioned vaccines have been developed based on the

141   prototype strain. It is possible to achieve a superior Omicron-neutralizing immunity

142   by variant vaccine boosters, especially when taking into account that some variants

143   such as Beta and Delta share some common key mutations with the Omicron variant.

144   Next, we developed various RBD-encoding mRNA vaccine candidates based on

145   prototype, Beta, Delta and Omicron strains as booster shots in BALB/c mice

146   following a single-dose prime injection with a prototype S-encoding mRNA vaccine

147   candidate, in order to assess the impact on humoral immunity against the Omicron

148   variant (Fig. 4A). Beta-Delta represents a vaccine combination of Beta and Delta

149   vaccine candidate with either half the other vaccine booster dose. The mice

150   immunized with a prime injection induced binding and neutralizing but no detectable

151   blocking antibodies against the Omicron variant, whereas all binding, neutralizing and

152   blocking antibodies against the prototype were detected in those mice (Fig. 4B-D).

153   The mice immunized with all types of booster shots had significantly elevated anti-

154   prototype strain binding, blocking, and neutralizing antibody titers, as well as anti-

155   Omicron binding and neutralizing antibody titers (Fig. 4B-D). In addition, all of the

156   booster groups induced significantly higher anti-Beta and anti-Delta binding antibody

157   titers compared with the no-booster control group (Fig. S5-6). However, only mice

158   immunized with Delta and Omicron boosters developed significantly higher anti-

159   Omicron blocking antibody titers compared with no booster control (Fig. 4C).

160   Notably, the prototype, Beta, Delta, Beta–Delta and Omicron vaccine boosters elicited

161   Omicron-neutralizing NT_{50} titers with values of 423, 1,202, 3,073, 1,548, and 7,710,

162   respectively, and only those elicited by Omicron booster were significantly higher

163   than those by the prototype booster (Fig. 4D), suggesting that Omicron-based mRNA

164   vaccine booster is superior to prototype-based mRNA vaccine booster in elevating

165   Omicron-neutralizing immunity.

166   **Discussion**

167   Since its emergence, the SARS-CoV-2 Omicron variant has been spreading at an

168   unprecedented speed. Consistent with its far more mutations in spike protein than

169   other variants, we demonstrated that the Omicron variant remarkedly escaped from

170   neutralizing antibody response elicited by full-course vaccinations of approved IAV

171 and AdV vaccines. To boost anti-Omicron response to sufficiently high titers so as to

172 provide some protection against Omicron infection, a booster shot may be necessary.

173 Here we showed that for prime vaccinations with IAV and AdV, a heterologous

174 vaccine booster with AdV and PRV, respectively, generated the highest increase in

175 Omicron-neutralizing antibody titers. Although the sample number in AdV booster

176 with prime IAV vaccinations was small (n=7), the robust increase in the Omicron-

177 neutralizing activity supports the heterologous AdV booster administration. Currently,

178 all of the approved vaccines have been developed based on the SARS-CoV-2

179 prototype strain. Using the mouse model, we further demonstrated that prototype

180 booster significantly increased the prototype vaccine-elicited Omicron-neutralizing

181 activity. Although the Beta and Delta variants harbor some identical or similar key

182 mutations to the Omicron variant, it is only the Omicron-based but not Beta- or Delta-

183 based vaccine booster that exhibited a significantly higher ability of improving

184 Omicron-neutralizing immunity. Taken together, our systematical investigation of

185 impact of various vaccine boosters on improving Omicron-neutralizing immune

186 response yielded important data to guide possible future booster shots to contain the

187 Omicron pandemic.

188   Structural comparisons have allowed us to classify RBD-targeted neutralizing

189 antibodies into four class groups (*19*). Class 1 and Class 2 antibodies bind on, or in

190 close proximity to the ACE2-binding footprint, and they can potently neutralize

191 viruses by blocking the interaction of RBD with ACE2, thereby preventing viral

192 attachment to host cells (*20*). As there are concentrated mutations on the ACE2-

193   binding footprint in Omicron RBD, these antibodies showed dramatic or complete

194   loss of the neutralizing activity against the Omicron variant (*17*). Thus, Omicron-

195   blocking titers of human serum specimens showed a substantial decrease compared

196   with the prototype-blocking titers. There are also class 3 and class 4 neutralizing

197   antibodies which bind distant from ACE2-binding site and do not block ACE2

198   interaction. These antibodies may destabilize the spike trimmer protein, and the

199   antibody epitopes are more conserved in the Omicron variant (*17*). In theory, these

200   antibodies are the main contributors of cross-neutralization against the Omicron

201   variant in prototype-vaccinated human serum. That also leads to a moderate decrease

202   in Omicron-neutralizing antibody titers in comparison with Omicron-blocking

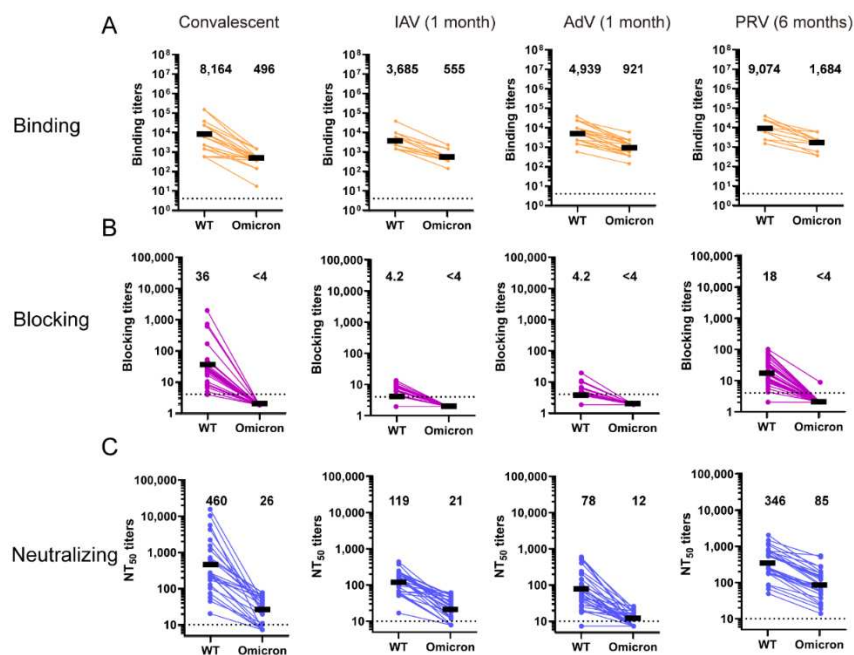203   antibody titers.

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Fig. 1. Serum binding, blocking and neutralizing antibody titers of convalescents and vaccinated individuals were markedly lower against Omicron compared to wild-type SARS-CoV-2. Samples included sera obtained from convalescents (n=25), one month after two-dose vaccination with IAV (n=30) or a single-dose AdV (n=30), and six months after three-dose vaccination with PRV (n=28). (A) Serum binding antibody titers were detected by ELISA assay. Coated antigen was wild type RBD or Omicron RBD. Dotted line indicates the limit of detection (>8). (B) hACE2-blocking antibody titers were detected by ELISA using wild-type or Omicron spike protein, which binds to human ACE2. The dotted line indicates the limit of detection (>4). (C) Pseudovirus neutralization titers, expressed as 50% neutralization dilution (NT_{50}). The pseudoviruses used in the study included both wild-type strain and Omicron. The dotted line indicates the limit of detection (>10). IAV represents inactivated vaccines (CoronaVac and BBIBP-CorV). PRV represents recombinant protein subunit vaccine (ZF2001). AdV represents adenovirus-vectored vaccine (Convidecia).
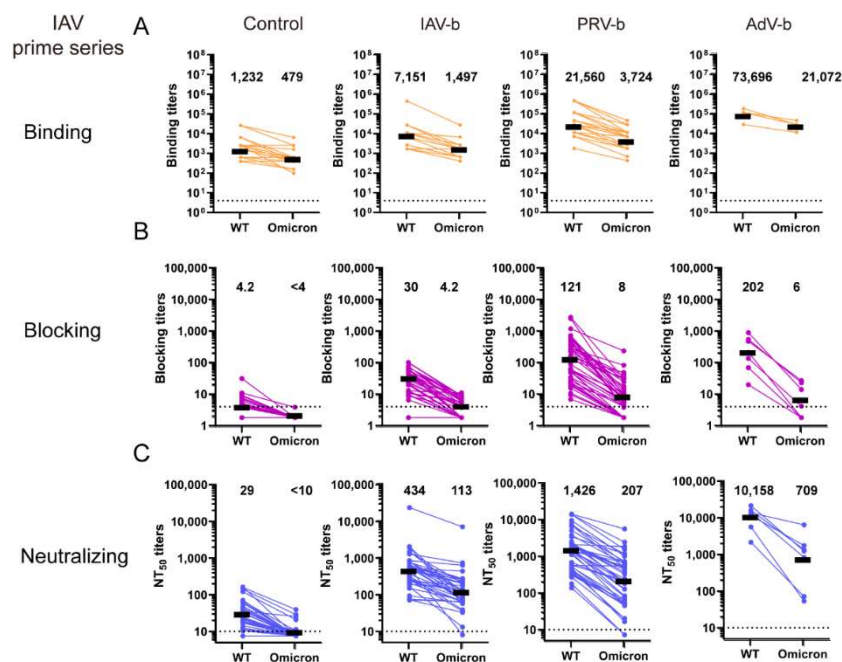
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Fig. 2. Serum binding, blocking and neutralizing antibody titers of no booster control and various vaccine boosters following prime vaccination with two-dose IAVs. Samples were obtained from participants without vaccine booster (n=42), with IAV (IAV-b, n=39), AdV (AdV-b, n=7), or PRV (PRV-b, n=45) boosters following two-dose prime vaccination with IAV 4-8 months earlier. (A) Serum binding antibody titers were detected by ELISA. Coated antigen was wild type RBD or Omicron RBD. Dotted line indicates the limit of detection (>8). (B) hACE2-blocking antibody titers were detected by ELISA using SARS-CoV-2 wild type and Omicron spike proteins which bind to human ACE2. The dotted line indicates the limit of detection (>4). (C) Pseudovirus neutralization titers, expressed as 50% neutralization dilutions (NT_{50}). The pseudoviruses used in the study included wild-type strain and Omicron. The dotted line indicates the limit of detection (>10). IAV represents inactivated vaccines (CoronaVac and BBIBP-CorV). PRV represents recombinant protein subunit vaccine (ZF2001). AdV represents adenovirus-vectored vaccine (Convidecia).
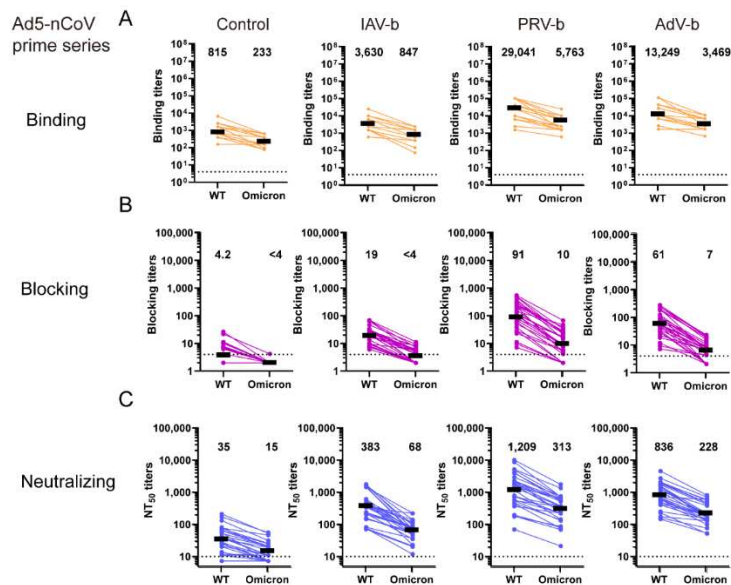
Fig. 3. Serum binding, blocking and neutralizing antibody titers of no booster control and various vaccine boosters following a single-dose prime vaccination with AdV. Samples were obtained from participants without vaccine booster (n=30), with IAV (IAV-b, n=30), AdV (AdV-b, n=30), or PRV (PRV-b, n=30) boosters following a single-dose prime vaccination with IAV 4–8 months earlier. (A) Serum binding antibody titers were detected by ELISA. Coated antigen was wild type RBD or Omicron RBD. The dotted line indicates the limit of detection (>8). (B) hACE2-blocking antibody titers were detected by ELISA using SARS-CoV-2 wild type and Omicron spike proteins which bind to human ACE2. The dotted line indicates the limit of detection (>4). (C) Pseudovirus neutralization titers, expressed as 50% neutralization dilutions (NT_{50}). The pseudoviruses used in the study included wild-type strain and Omicron. The dotted line indicates the limit of detection (>10). IAV represents inactivated vaccines (CoronaVac and BBIBP-CorV). PRV represents recombinant protein subunit vaccine (ZF2001). AdV represents adenovirus-vectored vaccine (Convidecia).
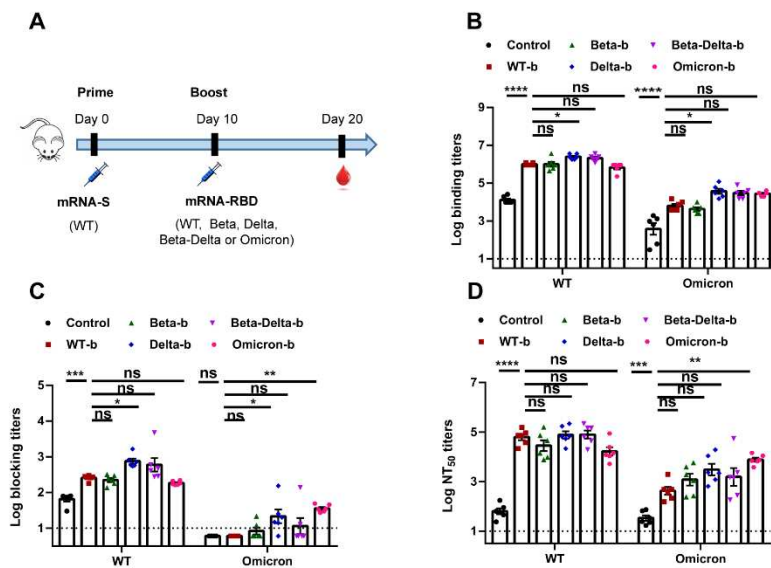
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Fig. 4. Investigation of various RBD-encoded mRNA vaccine boosters based on SARS-CoV-2 prototype, Beta, Delta, Beta plus Delta and Omicron following a single prime injection. Groups of BALB/c mice (n=6) received no booster or different vaccine booster shot following a prime immunization with wild type S-encoding mRNA vaccine candidate via intramuscular route. Mouse serum were obtained at 10 days following the booster injection. (A) Mice immunization schedule. (B) Mouse sera binding antibody titers were detected by ELISA. Coated antigen was wild type RBD or Omicron RBD. The dotted line indicates the limit of detection (>10). (C) hACE2-blocking antibody titers were detected by ELISA using SARS-CoV-2 wild type and Omicron spike proteins which bind to human ACE2. The dotted line indicates the limit of detection (>10). (D) Pseudovirus neutralization titers, expressed as 50% neutralization dilutions (NT_{50}). The pseudoviruses used in the study included wild-type strain and Omicron. The dotted line indicates the limit of detection (>10). P values were analyzed with One-Way ANOVA (ns, $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

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Materials and Methods

Human serum samples

Human serum specimens were collected at Peking Union Medical College Hospital,

Beijing Ditan Hospital, 309 Hospital of the Chinese People's Liberation Army,

Fangzhuang Community Health Service Center, and the Institute of Microbiology of

the Chinese Academy of Sciences (IMCAS). The samples were selected based on

availability, and the specimens were obtained from individuals of different genders

with no specific inclusion/exclusion criteria. Participants included convalescents from

COVID-19, participants who had received full-course vaccination with inactivated

vaccines, recombinant protein subunit vaccines, and adenovirus-vectored vaccines, as

well as various types of vaccine boosters.

Ethics statement

This study was reviewed and approved by IMCAS (APIMCAS2021159). This study

was conducted in strict accordance with the recommendations in the "Guide for the

Care and Use of Laboratory Animals" issued by the Ethics Committee of IMCAS.

Informed consent was obtained from all of the participants.

Cells, pseudoviruses, and animals

HEK293T (ATCC, CRL-1573) cells and Vero E6 cells (ATCC CRL-1586) were

cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented

with 10% fetal bovine serum (FBS). Pseudovirus of the SARS-CoV-2 wild type strain

and the Omicron variant were provided by Professor Weijin Huang from National

Institutes for Food and Drug Control. Specific-pathogen-free (SPF) BALB/c 6-8-

week-old female mice were purchased from Beijing Vital River Animal Technology

361   Co., Ltd (licensed by Charles River). All of the mice used in this study are were

362   housed and bred in a temperature-, humidity- and light cycle-controlled SPF mouse

363   facilities in IMCAS (20±2℃; 50±10%; light, 7:00-19:00; dark, 19:00-7:00).

364   Protein expression and purification

365   The objective recombinant protein sequences carried on the pCAGGS vector were

366   expressed via HEK293T cells. The supernatant of cell culture was collected five days

367   post-transfection. Initially, a Histrap excel 5 mL column (GE Healthcare) was used to

368   isolate proteins. The medium of samples collected from the Histrap columns was

369   substituted with PBS solution and then further purified with the Superdex 200 column

370   (GE Healthcare). Lastly, SDS-PAGE was performed to assess the purity of the

371   protein.

372   ELISA

373   SARS-CoV-2 RBD monomer proteins were coated with 50 mM carbonate-

374   bicarbonate buffer (pH 9.6) in Corning® 96-well Clear Polystyrene Microplates at 200

375   ng per well. The microplates were blocked by 5% skimmed milk at 37℃ for one hour,

376   and then the milk was discarded. The microplates were incubated with 100 μL two-

377   fold serially diluted mice serum at 37℃ for one hour. The HRP-labeled anti-mouse Fc

378   secondary antibody (Yeasen) was added after washing the microplates three times.

379   Then, 50 μL of 3, 3', 5, 5'-tetramethylbenzidine (Beyotime Biotechnology) was used

380   as a substrate and 50 μL of 2 M sulphuric acid was used to stop the reactions. The

381   absorbance was measured at 450 nm using a microplate reader (PerkinElmer). The

382   end-point antibody titers were defined as the highest dilution of the serum that

383   produced an optical absorption value (OD_{450}) 2.1 times higher than the background

384   value.

385   <u>hACE2-receptor-blocking assay</u>

386   hACE2-receptor-blocking antibodies were determined by ELISA. Corning® 96-well

387   Clear Polystyrene Microplates were coated with 20 μg/mL human ACE2 (hACE2)

388   protein overnight at 4°C. Serially diluted sera from groups of immunized mice or

389   humans was added into coated wells and then 50 ng/mL of histidine-tagged SARS-

390   CoV-2 S proteins was added into wells for two hours at 37°C. Meanwhile, a set of

391   negative control without S protein and a set of positive control without serum were

392   necessary. After incubation and washing five times, Anti-His-tag-HRP was added and

393   incubated for one hour. Then, 50 μL of 3, 3', 5, 5'-tetramethylbenzidine (Beyotime

394   Biotechnology) was used as a substrate and 50μL of 2M sulphuric acid was used to

395   stop the reactions. The absorbance was measured at 450 nm using a microplate reader

396   (Perkin Elmer). The reciprocal of the highest serum dilution that resulted in 50%

397   inhibition of receptor binding was used as the titer of the serum.

398   <u>Pseudovirus neutralization assay</u>

399   96 Well White Plates (WHB) were used to detect the neutralization potency. Sera

400   from groups of mice and humans were serially diluted in DMEM medium

401   supplemented with 10% FBS. Pseudoviruses were diluted to 2×10^4 $TCID_{50}$/mL using

402   DMEM (10% FBS), and then 50 μL of the diluted pseudoviruses was added to each

403   well. Meanwhile, a set of negative controls with only medium and a set of positive

404   controls with only pseudovirus were necessary. After incubation at 37°C for one

405   hour，2 × 10^4 Vero E6 per cell were added to each well to make a final volume of

406   200 μl, which was incubated for 24 hours in a 37℃, 5% CO2 incubator. After

407   incubation, the supernatant in the wells was discarded and 100 μL of luciferase

408   detection reagent (PerkinElmer, Inc.) was added. The reaction was shaken at room

409   temperature for two minutes and the fluorescence values were read in a

410   chemiluminescence detector (Promega GloMax). The neutralization NT_{50} titer was

411   defined as the fold-dilution of serum necessary for 50% inhibition of luciferase

412   activity in comparison with virus control samples.

413   <u>mRNA production</u>

414   mRNA was produced using T7 RNA polymerase on linearized plasmids (synthesized

415   by Genescript) encoding codon-optimized SARS-CoV-2 S6P protein or prototype,

416   Beta, Delta, or Omicron RBD glycoprotein (residues 319-541). The mRNA was

417   transcribed to contain a 104 nucleotide-long poly(A) tail, and 1-methylpseudourine-

418   5′-triphosphate was used instead of UTP to generate modified nucleoside-containing

419   mRNA. The mRNA was purified by overnight LiCl precipitation at -20℃, centrifuged

420   at 18,800×g for 30 min at 4℃ to pellet, washed with 75% EtOH, centrifuged at

421   18,800×g for 1 min at 4℃, and resuspended in RNase-free water. The purified mRNA

422   was analyzed by agarose gel electrophoresis and stored at -80℃ until use.

423   <u>Lipid-nanoparticle encapsulation of mRNA</u>

424   mRNA was encapsulated in LNPs using a self-assembly process in which an aqueous

425   solution of mRNA at pH=4.0 was rapidly mixed with a solution of lipids dissolved in

426   ethanol. LNPs used in this study contained an ionizable cationic lipid,

427   phosphatidylcholine, cholesterol, and PEG-lipid at a ratio of 50:10:38.5:1.5 mol/mol

428   and were encapsulated at an mRNA to lipid ratio of around 0.05 (wt/wt). The

429   formulations were then diafiltrated against 100 x volume of Phosphate Buffered

430   Saline (PBS) through a tangential-flow filtration (TFF) membrane with 10 kD

431   molecular weight cut-offs (Sartorius Stedim Biotech), concentrated to a required

432   concentration, passed through a 0.22μm filter, and stored at 4°C with a concentration

433   of RNA of about 1 mg/mL.

434   Animal experiments

435   For immunization, 6-8-week-old female BALB/c mice were primarily vaccinated with

436   4 μg S-encoding mRNA vaccine candidate via intramuscular (i.m.) route. At 10 days

437   following primary immunization, booster injections with PBS as negative control or

438   different variant RBD-encoding mRNA vaccine candidates were administered with a

439   dose of 4 μg. Serum samples were collected at 10 days following booster vaccination,

440   inactivated at 56°C for 30 min, and stored at -80°C until use.

441   Statistical analysis

442   All of the data are expressed as the mean ± standard error of the mean. For all of the

443   analyses, P values were obtained from Student's t-test (unpaired, two tailed) or One-

444   way ANOVA test. All of the graphs were generated with GraphPad Prism version 7.0
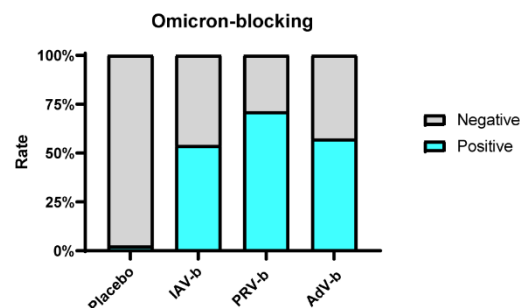
445   software.

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Fig. S1. Rate of positive samples for hACE2 blocking antibody detection against

Omicron, relative to Fig. 2B. Samples were obtained from participants without

vaccine booster (n=42), with IAV (IAV-b, n=39), AdV (AdV-b, n=7), or PRV (PRV-b,

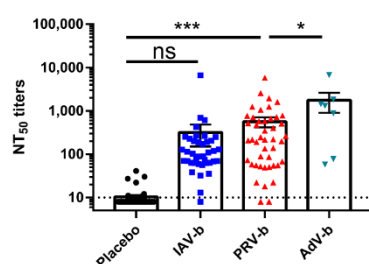n=45) boosters following two-dose prime vaccination with IAV 4–8 months earlier.

454

Fig. S2. Neutralization titers against Omicron pseudovirus of non-boosted and

boosted with different vaccines following previously vaccinated with IAVs,

relative to Fig. 2C.

Samples were obtained from participants without vaccine booster (n=42), with IAV

(IAV-b, n=39), AdV (AdV-b, n=7), or PRV (PRV-b, n=45) boosters following two-

dose prime vaccination with IAV 4–8 months earlier. The values were representative

of mean $\pm$ SEM. P values were analyzed with Student's t-test (ns, $p>0.05$, $*p<0.05$,

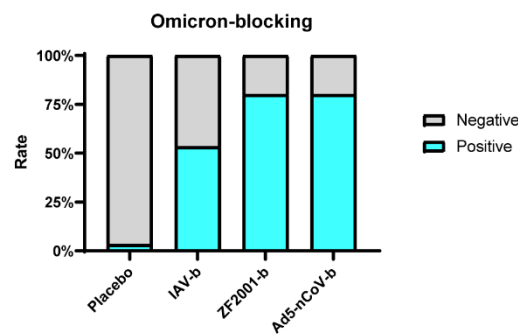$**p<0.01$, $***p<0.001$, and $****p<0.0001$).

463

Fig. S3. Rate of positive samples for hACE2 blocking antibody detection against Omicron, relative to Fig. 3B. Samples were obtained from participants without vaccine booster (n=30), with IAV (IAV-b, n=30), AdV (AdV-b, n=30), or PRV (PRV-b, n=30) boosters following a single-dose prime vaccination with IAV 4–8 months earlier.
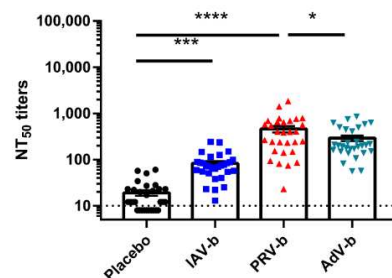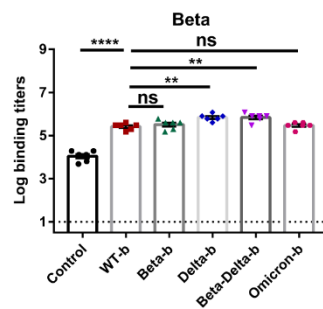

469

Fig. S4. Neutralization titers against Omicron pseudovirus of non-boosted and boosted with different vaccines following previous vaccination with AdV, relative to Fig. 3C.

Samples were obtained from participants without vaccine booster (n=30), with IAV (IAV-b, n=30), AdV (AdV-b, n=30), or PRV (PRV-b, n=30) boosters following a single-dose prime vaccination with IAV 4–8 months earlier. The values represent mean ± SEM. P values were analyzed with t-test (ns, $p > 0.05$, *$p < 0.05$, **$p < 0.01$,

477   ***p<0.001, and ****p<0.0001).
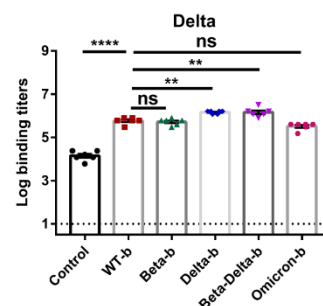

478

479   **Fig. S5. Beta-binding titers of various RBD-encoded mRNA vaccine boosters**

480   **based on SARS-CoV-2 prototype, Beta, Delta, Beta plus Delta and Omicron**

481   **following a single prime injection, relative to Fig. 4.** The dotted line indicates the

482   limit of detection (>10). P values were analyzed with One-Way ANOVA (ns, p>0.05,

483   *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001).

484

485   **Fig. S6. Delta-binding titers of various RBD-encoded mRNA vaccine boosters**

486   **based on SARS-CoV-2 prototype, Beta, Delta, Beta plus Delta and Omicron**

487   **following a single prime injection, relative to Fig. 4.** The dotted line indicates the

488   limit of detection (>10). P values were analyzed with One-Way ANOVA (ns, p>0.05,

489   *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001).

490

Table S1 Characteristics of participants

	Group	Number	Male (%)	Female (%)	Age years mean (SD)	Immunization Regimen
Infection	Convalescent	25	15 (60%)	10 (40%)	37.3 (14.4)	No
Full-course vaccinations	IAV	30	13 (43%)	17 (57%)	67.8 (7.5)	0, 1 month
	AdV	30	9 (30%)	21 (70%)	37.5 (7.6)	0 month
	PRV	28	10 (36%)	18 (64%)	32.0 (8.0)	0, 1, 2 months
Prime vaccinations with two doses of IAV	no booster	42	12 (29%)	30 (71%)	37.1 (8.0)	0, 1 month
	IAV booster	39	10 (26%)	29 (74%)	37.9 (11.0)	0, 1, 4-8 months
	PRV booster	45	13 (29%)	32 (71%)	41.1 (8.2)	0, 1, 4-8 months
	AdV booster	7	3 (43%)	4 (57%)	33.1 (4.5)	0, 1, 4-8 months
Prime vaccinations with one dose of AdV	no booster	30	6 (20%)	24 (80%)	36.0 (7.2)	0 month
	IAV booster	30	6 (20%)	24 (80%)	35.0 (4.2)	0, 4-8 months
	PRV booster	30	7 (23%)	23 (77%)	38.7 (1.5)	0, 4-8 months
	AdV booster	30	7 (23%)	23 (77%)	35.8 (8.9)	0, 4-8 months

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