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BRIEF REPORT

Hamsters Protected from SARS-CoV-2 Delta Variant Challenge after Two Doses of Adjuvanted SARS-CoV-2 Recombinant Spike Protein (S-2P) and One Dose of Beta S-2P

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern negatively impact the effectiveness of vaccines. In this study, we challenge hamsters with the delta variant after 2- or 3-dose inoculations with SARS-CoV-2 vaccines constructed from stabilized prefusion spike proteins (S-2P) of Wuhan (W) and beta (B) variants. Compared to 3 doses of W S-2P, 2 doses of W S-2P followed by a third dose of B S-2P induced the highest neutralizing antibody titer against live SARS-CoV-2 virus and enhanced neutralization of omicron variant pseudovirus. Reduced lung live virus titer and pathology suggested that all vaccination regimens protect hamsters from SARS-CoV-2 delta variant challenge.

Keywords. SARS-CoV-2 vaccine; COVID-19 vaccine; subunit vaccine; MVC-COV1901; hamster challenge study; variant of concern.

Despite mass vaccination programs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), variants of concern (VoCs) such as the delta and omicron variants have become dominant strains [1]. These VoCs have increased transmission rates, reduced in vitro neutralizing capability and clinical effectiveness of currently available vaccines, and are more resistant to neutralization by convalescent and vaccine-induced antibodies [2, 3]. The most current data point towards booster vaccinations for enhancing immune response and improving effectiveness against the VoCs [4, 5].

Medigen's MVC-COV1901 is a subunit vaccine based on a stabilized prefusion spike protein (S-2P) adjuvanted with CpG 1018 and aluminum hydroxide, which has been approved for emergency use in Taiwan [6, 7]. We have previously shown that 2 doses of adjuvanted S-2P induced neutralizing antibodies against SARS-CoV-2 variants with a tendency to higher immunogenicity at higher dose levels [8]. A third dose of MVC-COV1901 in the phase 1 subjects was also found to improve neutralization response against the omicron variant [9]. The current study expands on our previous findings by investigating the immunogenicity of a third-dose variant-specific booster against VoCs.

METHODS

Animals and Ethics Statements

Female golden Syrian hamsters aged 8–10 weeks at study initiation were obtained from the National Laboratory Animal Center (Taipei, Taiwan). Animal immunizations were conducted in the Testing Facility for Biological Safety, TFBS Bioscience Inc, Taiwan. Seven weeks after the final immunization and after serum sampling, the animals were transferred to Academia Sinica, Taiwan, to allow for 1 week of acclimatization before SARS-CoV-2 challenge. All procedures in this study involving animals were conducted in a manner to avoid or minimize discomfort, distress, or pain to the animals and were carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org/). All animal work in the current study was reviewed and approved by the Institutional Animal Care and Use Committee, with animal study protocol approval number TFBS2020-019, and Academia Sinica (approval number, 20-06-1483).

Immunization and Challenge of Hamsters

The study design is outlined in Supplementary Figure 1. The hamsters were split into 6 groups (A to F) with n = 10 for each group (Supplementary Table 1). Vaccine was administered to hamsters via intramuscular injection in quadriceps femoris muscle of left and right legs (50 μL each leg for a total of 100 μL per dose). All immunizations with S-2P were adjuvanted with 150 μg of CpG 1018 and 75 μg of alum. Serum samples were collected 5 weeks after the final immunization and immunogenicity was determined by neutralization assay with SARS-CoV-2 virus and the variants. Approximately 3 weeks after the serum sampling (53 days after the final immunization), hamsters were challenged with the SARS-CoV-2 delta variant (TCDC [Taiwan Centers for Disease Control] No.

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1144) and then sacrificed at 3 days postinfection (dpi) (n = 5 per group) or 6 dpi (n = 5 per group) for analyses of lung viral loads and lung 50% tissue culture infectious dose ($TCID_{50}$). Body weights of individual hamsters were tracked daily up to the time of sacrifice. After euthanasia, necropsy was performed and lungs of sacrificed hamsters were harvested, prepared, and sectioned and evaluated with a lung histopathological scoring system as previously described [10].

Laboratory Methods

SARS-CoV-2 virus strains, including Wuhan prototype strain (hCoV-19/Taiwan/4/2020, GISAD EPI_ISL_411927), alpha (B.1.1.7, hCoV-19/Taiwan/792, GISAD EPI_ISL_1381386), beta (B.1.351, hCoV-19/Taiwan/1013), gamma (P.1, hCoV-19/Taiwan/906), and delta (B.1.617.2, hCoV-19/Taiwan/1144) variants, were used in a live virus neutralization assay as described previously with results expressed as 50% neutralizing titer (NT_{50}) [7]. Pseudovirus neutralization assays with lentivirus pseudotyped with S proteins of the Wuhan strain or omicron variant were conducted as previously described with results expressed as 50% inhibition dilution (ID_{50}) [6, 8]. Supplementary Table 2 lists the mutations in the spike sequences used for the construction of omicron pseudovirus. Quantifications of lung viral load by real-time polymerase chain reaction (PCR) and $TCID_{50}$ assays were performed as previously reported [10].

Statistical Analysis

Statistical analysis was performed with Prism 6.01 (GraphPad). The comparisons of neutralizing antibody titers were performed using Kruskall-Wallis test with corrected Dunn's multiple comparisons test, 2-way ANOVA with Dunnett multiple comparison test, and unpaired Mann-Whitney *U* test.

RESULTS

Induction of Neutralizing Antibodies Against VoCs in Hamsters Immunized With 2 Doses of Adjuvanted S-2P Vaccines Based on the Wuhan Strain (W S-2P), Beta Variant (B S-2P), or Combinations of Both

We first examined the neutralizing antibody titers from hamsters immunized with 2 doses of 1 μg W S-2P adjuvanted with CpG 1018 and aluminum hydroxide (group A, W + W). Compared to the Wuhan strain (WT), the alpha, beta, gamma, and delta variants showed 3.79-, 13.30-, 11.39-, and 2.97-fold reductions in neutralizing titer levels, respectively, at 5 weeks after the second dose (Figure 1A). This demonstrated that 2 doses of W S-2P were relatively effective in stimulating neutralizing antibody against the alpha and delta variants but were less effective against the beta and gamma variants.

At the same time, we examined the neutralizing antibody titers from hamsters immunized with 2 doses of 1 μg of the adjuvanted beta variant S-2P (group B, B + B). Two doses of the adjuvanted B S-2P induced satisfactory immune response against the WT and beta variant but were less effective against the alpha, gamma, and delta variants (Figure 1A). We also explored the neutralizing antibody responses to bivalent vaccine consisting of W and B S-2Ps in group C hamsters, that is (W + B) + (W + B). The bivalent vaccine induced geometric mean titersr (GMTs) against the WT, alpha, and delta variants similar to those of the W + W group and induced higher GMTs against the beta and gamma variants than the W + W group (Figure 1A).

Induction of Neutralizing Antibodies Against VoCs in Hamsters Immunized With 2 Doses of W S-2P and a Third Dose of W S-2P or B S-2P

Next, we immunized hamsters with a third dose of adjuvanted W S-2P (group D, W + W + W) and we analyzed neutralizing titers 5 weeks later. Compared to the WT (Figure 1A), neutralizing titers against the alpha, beta, gamma, and delta variants were reduced by 3.54-, 15.30-, 11.41-, and 3.14-fold, respectively. Compared to group A, the neutralizing antibody GMT in group D against VoCs increased with the additional third dose. We also explored the possibility of using the adjuvanted beta variant version of S-2P as the third dose in group E (W + W + B). Compared to WT, this resulted in reductions of neutralizing titers against the alpha, beta, gamma, and delta variants of 3.52-, 6.42-, 5.09-, and 1.85-fold, respectively. Compared to the other groups, the W + W + B regimen resulted in the highest neutralization titers against the WT and all of the VoCs tested, especially against the delta variant. Overall, the neutralizing titers were lowest for the beta and gamma variants and regardless of the treatment group (Figure 1A).

In the omicron pseudovirus neutralization assay, the GMTs against omicron were reduced dramatically in all groups, but group E showed less reduction (6.8-fold) than group D (17.8-fold) (Figure 1B). Boosting with the beta variant S-2P (group E) increased ID_{50} GMT against WT and omicron by 1.5 times and 3.8 times, respectively, compared to group D (Figure 1B). Thus, live virus and pseudovirus assays show that 2 doses of W S-2P followed by a booster dose of B S-2P increase immunity against VoCs, including the omicron variant, better than 3 doses of W S-2P.

Protection of Hamsters From Delta Variant Challenge After Immunization With 2 Doses of W S-2P Alone or Followed by a Third Dose of W S-2P or B S-2P

Hamsters in all treatment groups (groups A to E) initially lost weight for up to 3 dpi but recovered and did not show significant weight loss by 6 dpi (Figure 2A). In contrast, the adjuvant control group showed a steady decline in weight that coincided with the high lung viral titer and RNA load in this group (Figure 2A–2C). At 3 dpi, lung viral RNA levels in the treatment groups were lower than in the adjuvant control group, but the difference was only significant in group E ($P < .01$; Figure 2B). In contrast, by 6 dpi the viral RNA levels in all groups were significantly ($P < .05$) lower than in the adjuvant control group. $TCID_{50}$ levels were significantly lower

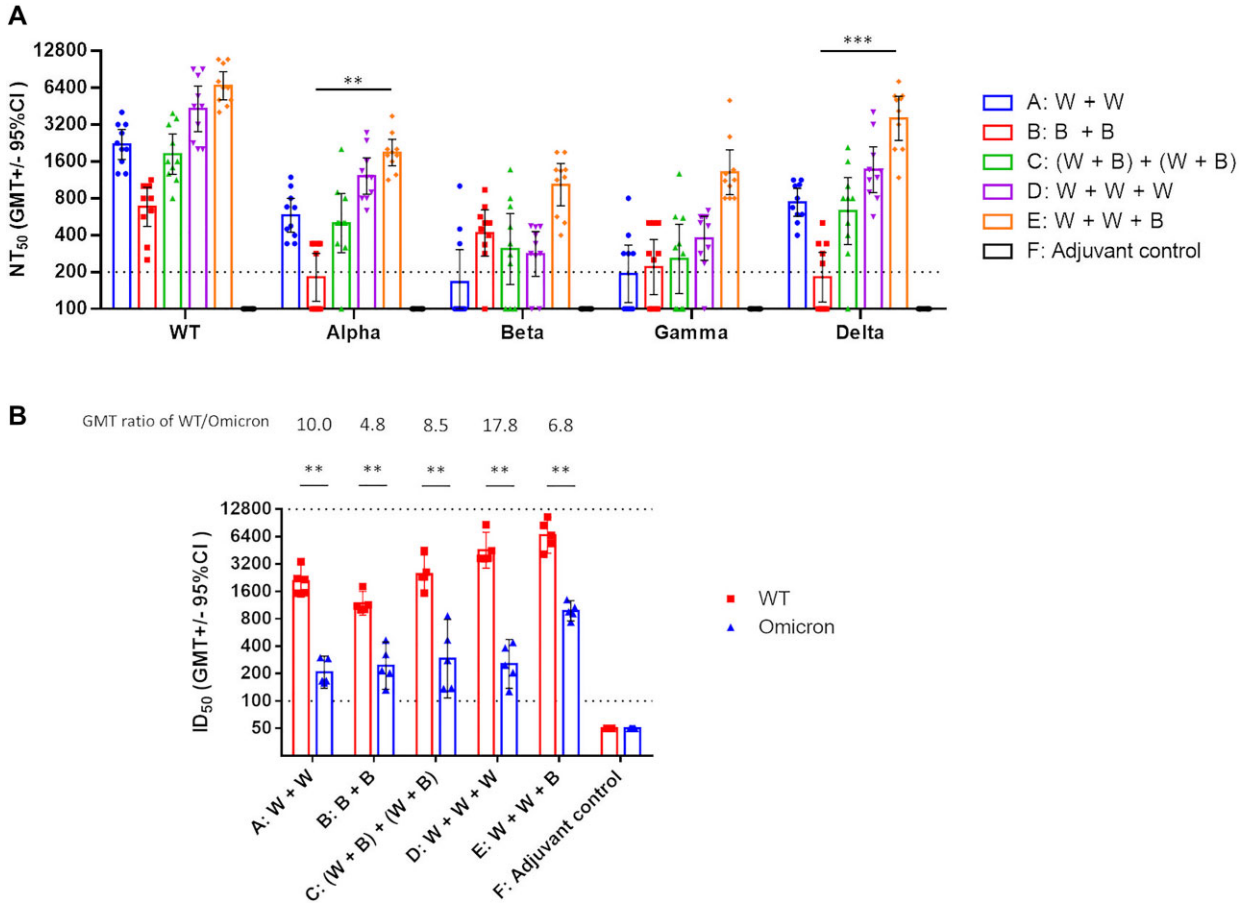

Figure 1. Neutralizing antibody titers by live SARS-CoV-2 neutralization assay for hamsters 5 weeks after the final immunization. Hamsters were immunized as in Supplementary Figure 1. *A*, Five weeks after the final immunization (second immunization for groups A, B, and C; third immunization for groups D, E, and F), serum samples were taken for neutralization assays against live SARS-CoV-2 Wuhan strain and alpha, beta, gamma, and delta variants. Bars indicate NT_{50} GMT with individual values displayed as symbols and error bars showing the 95% confidence intervals. Dotted line indicates the starting dilution (200) and all values below 200 are tabulated as 1-00. *B*, Analysis of serum samples from 10 hamsters per group, with each of 2 hamsters pooled to form a sample size of n = 5 per group. The pooled samples were tested against WT and omicron variant by pseudovirus neutralization assay. Vertical bars indicate the ID_{50} GMT with individual ID_{50} values displayed as symbols and error bars showing the 95% CIs. Dotted lines indicate the starting dilution (100) and the final dilution (12 800) for the assay. GMT ratio between WT and omicron is shown above the corresponding bars. Statistical significance was calculated with Kruskal-Wallis test with corrected Dunn's multiple comparisons test. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. Abbreviations: B, beta variant S-2P; CI, confidence interval; GMT, geometric mean titer; ID_{50}, 50% inhibition dilution; NT_{50}, 50% neutralizing titer; S-2P, stabilized prefusion spike protein vaccine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; W + B, bivalent mixture of Wuhan and beta variant S-2Ps; W, Wuhan strain S-2P; WT, wild type.

($P < .05$) at 3 dpi in all treatment groups relative to the adjuvant control (Figure 2C). There were no differences in histopathology scores at 3 dpi between control and experimental groups (Figure 2D). However, at 6 dpi, the adjuvant control group had significantly ($P < .01$) increased lung pathology, including extensive and severe immune cell infiltration, hemorrhage, and diffuse alveolar damage, compared to groups receiving 3 doses of S-2P (ie, groups D and E; Figure 2D and Supplementary Figure 2).

DISCUSSION

Here we report live virus and pseudovirus neutralization titers elicited in hamsters by 5 combinations of adjuvanted Wuhan and beta S-2P vaccines given up to 3 times. We found that 2 doses of W S-2P followed by a dose of B S-2P induced the highest neutralizing antibody titer and broadest spectrum against all VoCs tested. The same vaccination regime also significantly increased neutralizing antibody titer against omicron variant pseudovirus (Figure 1B). All 5 vaccination regimens protected hamsters from weight loss and reduced viral load after infection with delta variant (Figure 2). Interestingly, while group B had a relatively poor antibody response against the delta variant, the protection offered by this regimen against weight loss was comparable to other groups in which the hamsters did not experience any weight loss or increase in lung pathology (Figure 2). This suggests that, apart from neutralizing antibodies, protection from disease could also be attributed to innate and cellular

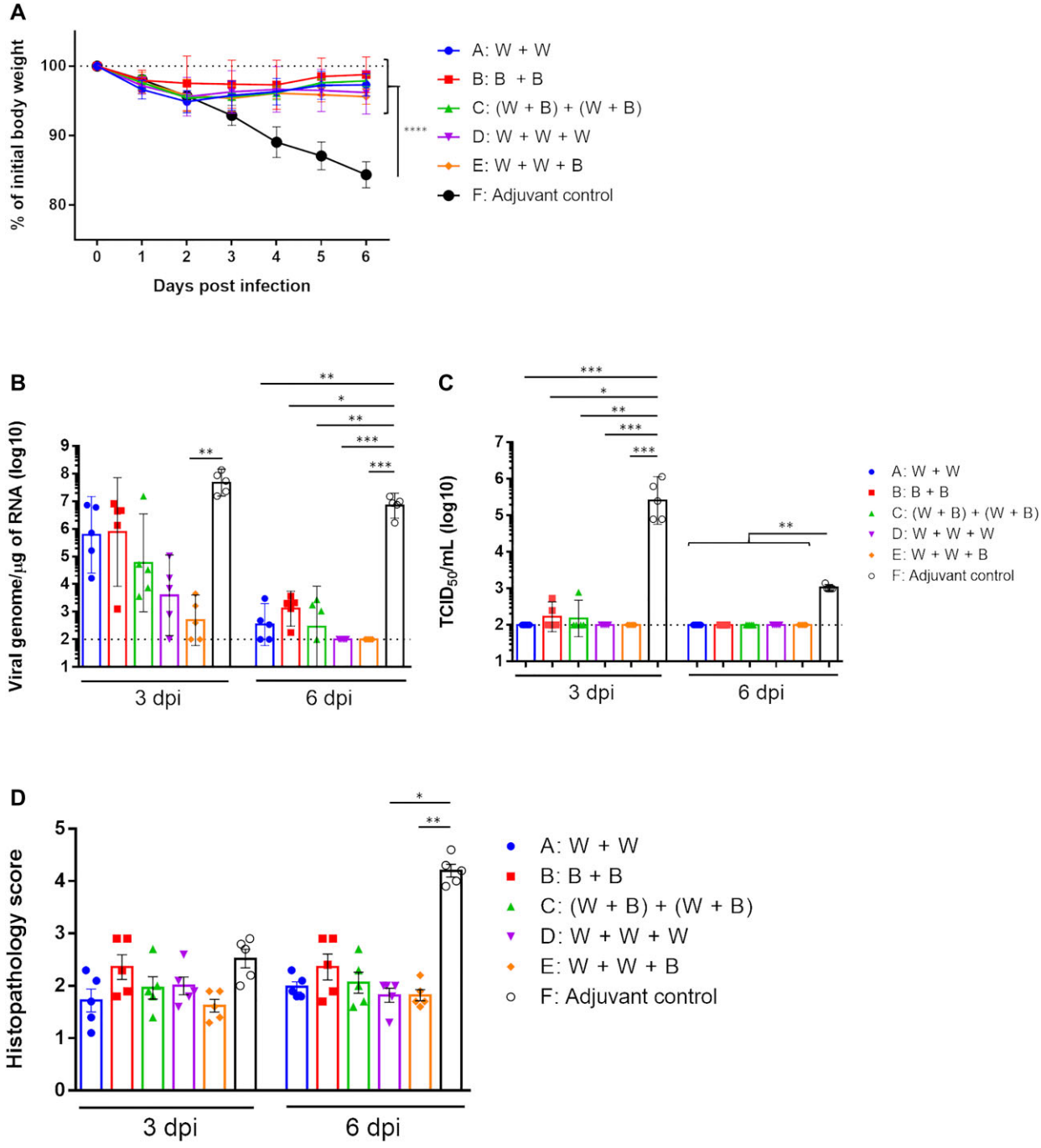

Figure 2. Challenge of hamsters with SARS-CoV-2 delta variant 8 weeks after the final immunization. Ten hamsters were included per vaccine treatment group, with 5 sacrificed at 3 dpi and 5 sacrificed at 6 dpi. *A*, The body weights of individual hamsters (n = 5 per group) were tracked daily up to the time of sacrifice at 6 dpi. Results are shown as average for each group as percent of initial body weight at day 0 (day of challenge). *B* and *C*, Hamsters were sacrificed at 3 or 6 dpi and lung tissue samples were collected for viral load determination by quantitative PCR of viral genome RNA (*B*) and $TCID_{50}$ assay for virus titer (*C*). Results are presented as geometric mean values with individual hamster values shown and with error bars representing 95% confidence intervals. Dotted line indicates limit of detection (100) and all values below the limit of detection are tabulated and calculated as 100. *D*, Lung sections were prepared and stained at 3 or 6 dpi, and histopathology scores were calculated. Results are presented both as individual values (n = 5) and mean with error bars representing standard error of the mean. Statistical significance was calculated by (*A*) 2-way ANOVA with Dunnett multiple comparison test with adjuvant only as a control, and (*B* and *C*) Kruskal-Wallis corrected Dunn's multiple comparisons test. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. Abbreviations: B, beta variant S-2P; dpi, days postinfection; PCR, polymerase chain reaction; S-2P, stabilized prefusion spike protein vaccine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; $TCID_{50}$, 50% tissue culture infectious dose; W + B, bivalent mixture of Wuhan and beta variant S-2Ps; W, Wuhan strain S-2P.

immunity as previously demonstrated in a ChAdOx1 nCoV-19 clinical study [11].

As vaccines induce polyclonal neutralizing antibodies, they could be cross-reactive to different SARS-CoV-2 variants. SARS-CoV-2 vaccine could induce broadly neutralizing antibodies targeting the N-terminal domain and residues in the receptor-binding domain that are conserved across SARS-CoV-2 variants [12]. Mechanistically, this could be because a booster of beta variant S-2P after 2 doses of W S-2P selects for B cells that produce antibodies against conserved epitopes between variants, and elicits a broadly reactive T-cell immune response, as shown in a study with recipients receiving a variety of vaccines [13]. The inability of RNA amplification assay to distinguish between replicating virus and inactivated virus may explain the discrepancy between detectable levels of viral RNA and undetectable $TCID_{50}$ levels at 3 dpi, as observed in our previous hamster study [10].

One limitation of this study is that we have not tested in vivo protection by our vaccine with VoCs other than the delta variant. Second, the natural course of infection in hamsters results in eventual convalescence, and so the model does not permit evaluation of mortality or severe disease end points, and are inadequate as models for omicron infection due to limited weight loss and lower viral load [14]. The lung histopathology scoring system we used in our animal model also did not distinguish between different levels of lung damage caused by different degrees of viral replication in the lung, and no immunohistochemistry was done to visualize the presence of viral antigens to extend on our viral RNA detection and $TCID_{50}$ assays. Finally, hamster T-cell responses were not evaluated in this study, but a nonhuman primate challenge study at the US National Institutes of Health has shown that adjuvanted W S-2P induced Th1-biased response with no detectable CD8 T cell response (Robert Seder, personal communication).

Despite these limitations, it is clear that our antibody neutralization results with boosters, described here, mirror other studies. For example, administration of either mRNA-1273 (original) or mRNA1273-351 (beta variant) as a third dose exponentially boosted immunogenicity against beta, gamma, and delta variants compared to 2 doses of mRNA1273 [15]. Other published data also support the notion that boosting with vaccines can generate anti-omicron neutralizing response that cannot be achieved by primary series of vaccination [2–4]. The findings from this study support the further evaluation of both the original and beta variant S-2P vaccines as booster doses for individuals fully vaccinated with MVC-COV1901 or other approved vaccines.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. T.-Y. K., C.-C. W., W.-H. T, and J. C. produced the Wuhan and beta variant versions of S-2P antigens and pseudoviruses used in the study. T.-Y. K., C.-E. L., Y.-J. L., M.-Y. L., C.-C. W, W.-H. T., Y.-S. C., and C. C. designed the study and experiments. Y.-J. L. and Y.-S. C. supervised the experiments at TFBS Bioscience and Academia Sinica. Y.-J. L., M.-Y.-L., Y.-S. C., and L. T.-C. L. analyzed the results. M.-Y. L., J. D. C., P. T., Y.-S. C., and L. T.-C. L. drafted the manuscript. All authors reviewed and approved of the final version of the manuscript.

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Potential conflicts of interest. C. C., T.-Y. K., C.-C. W., W.-.H. T, C.-E. L., Y.-J. L., and M.-Y. L. are coinventors for US provisional patent applications 63/240,408, 63/240,080, 63/248,189. and 63/251,741. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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