Protection of Hamsters Challenged with SARS-CoV-2 Variants of Concern by
Two Doses of MVC-COV1901 Vaccine Followed by a Single Dose of Beta Variant
Version of MVC-COV1901

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Abstract

The current fight against COVID-19 is compounded by the Variants of Concern (VoCs), which can diminish the effectiveness of vaccines, increase viral transmission and severity of disease. MVC-COV1901 is a protein subunit vaccine based on the prefusion SARS-CoV-2 spike protein (S-2P) adjuvanted with CpG 1018 and aluminum hydroxide. Here we used the Delta variant to challenge hamsters innoculated with S-2P based on the ancestral strain or the Beta variant in two-dose or three-dose regimens. Two doses of ancestral S-2P followed by the third dose of Beta variant S-2P was shown to induce the highest neutralizing antibody titer against live SARS-CoV-2 of the ancestral strain as well as all VoCs. All regimens of vaccination were able to protect hamsters from SARS-CoV-2 Delta variant challenge and reduce lung live virus titer. Three doses of vaccination significantly reduced lung viral RNA titer, regardless of using the ancestral or Beta variant S-2P as the third dose. Based on the immunogenicity and viral challenge data, two doses of ancestral S-2P followed by the third dose of Beta variant S-2P could induce broad and potent immune response against the variants.
Introduction

As of September 2021, the COVID-19 pandemic shows no sign of abating despite over five billion doses of vaccines administered around the world, partly due to the emergence of VoCs. The WHO so far has listed four VoCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), and five VoIs: Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), and Mu (B.1.621) [1]. The VoCs are known to reduce the in vitro neutralizing capability of currently available vaccines through mutations on the spike protein, especially in the receptor binding domain (RBD) [2-4]. These in vitro data also translate to the clinical data, with reports of considerably decreased vaccine efficacy in particular against the Beta and Delta variants [5-8]. Compared to redesigning the vaccine or creating a SARS-CoV-2 universal vaccine, the use of a booster dose may be currently the best way to protect against the variants [9-11] However, the use of boosters has led to heated political debates at a time where vaccine parity is laid bare between vaccine stockpiling in developed nations and critical lack of vaccine in developing nations [12-14].

MVC-COV1901 is a protein subunit vaccine based on the S-2P protein adjuvanted with CpG 1018 and aluminum hydroxide and has been shown to be safe and highly immunogenic in preclinical studies and clinical trials [15-18]. The vaccine has been approved for emergency use in Taiwan and is given intramuscularly as two doses separated by four weeks [19]. We have previously shown that two doses of MVC-COV1901 could induce neutralizing antibodies against SARS-CoV-2 variants with increasing tendency of higher immunogenicity at higher dose level [20]. In the same study we found that a third dose of S-2P in rats was able to increase neutralizing titer against the Beta variant compared to two doses of S-2P [20]. For the current study we expand on our previous findings to investigate the immunogenicity of third dose booster against the VoCs.

Results

Induction of neutralizing antibodies against VoCs in hamsters immunized with two doses of S-2P derived from original MVC-COV1901, Beta variant, or a bivalent combination of both.
We have previously shown that there was an approximately 7-fold reduction of neutralizing antibody
titer against the Beta variant in vaccinees’ sera from the MVC-COV1901 phase I trial [20]. As a strategy to
combat against the Beta variant, we have developed the Beta variant version of S-2P adjuvanted with 750 μg
of CpG 1018 and 375 μg of aluminum hydroxide. We have previously established that two doses of one-fifth
amount of low or high human doses of MVC-COV1901 were sufficient in protecting hamsters from
SARS-CoV-2 infection [15]. Since the Delta variant instead of the Beta variant has become prevalent
world-wide, we investigated the protective effects of MVC-COV1901, its Beta variant version of S-2P, or the
ancestral/Beta S-2P bivalent vaccine on hamsters challenged with the Delta variant. The experimental design
is outlined in Figure 1, where five groups of hamsters received different regimens of S-2P derived from the
ancestral and/or Beta variant, while a sixth group was administered with adjuvant alone. We first examined
the neutralizing antibody titers from hamsters immunized with two doses of one-fifth amount of low dose
MVC-COV1901 (i.e. 1 μg S-2P adjuvanted with 150 μg CpG 1018 and 75 μg aluminum hydroxide), Group A
(W + W), against all VoCs. As shown in Figure 2, at five weeks after the second injection of Group A
hamsters, reciprocal neutralizing antibody titer 50 (NT₅₀) GMT of 2201, 581, 166, 193, and 742 against the
ancestral strain, Alpha, Beta, Gamma, and Delta variants were obtained, respectively. Compared to the
neutralizing titer against the ancestral strain, that against the Alpha, Beta, Gamma, and Delta variants showed
3.79-, 13.30-, 11.39-, and 2.97-fold reduction, respectively. This demonstrated that two doses of S-2P derived
from ancestral strain was relatively effective against the Alpha and Delta variants. However, the effectiveness
was significantly reduced against the Beta and Gamma variants.

We next examined the neutralizing antibody titers from hamsters immunized with two doses of 1 μg of
the Beta variant version of S-2P combined with 150 μg CpG 1018 and 75 μg aluminum hydroxide, Group B
(B + B). Figure 2 shows that two doses of the adjuvanted Beta variant S-2P induced lower GMT against the
ancestral strain but increased against the Beta variant compared to group A, which were 681 and 417,
respectively. However, compared to group A, the neutralizing titers of this regimen were lower against the
Alpha and Delta variants, which were 181 and 182, respectively.
We also explored the neutralizing antibody responses of bivalent vaccine in Group C hamsters [(W + B) + (W + B)]. The bivalent vaccine induced a similar degree of neutralizing antibody titers against the ancestral strain, Alpha, and Delta variants to that of the W+W group. This combination fared better against the Beta and Gamma variants than that of the W+W group. However, several individual hamsters did not show any neutralization titer against these variants.

**Induction of neutralizing antibodies against VoCs in hamsters immunized with two doses of MVC-COV19 combined with a third dose of MVC-COV1901 or its Beta variant version of S-2P.**

We previously found that neutralizing antibody titers against the Beta variant were increased substantially in rats receiving three doses rather than two doses of MVC-COV1901 [20]. Therefore, we immunized hamsters with a third dose of one-fifth amount of MVC-COV1901, Group D (W + W + W), to examine antibody responses against VoCs. As shown in Figure 2, at five weeks after the third injection of Group D hamsters, NT50 GMTs were 4302, 1217, 281, 377, and 1368 against the ancestral strain, Alpha, Beta, Gamma, and Delta variants, respectively. The neutralizing titer against the Alpha, Beta, Gamma, and Delta variants had 3.54-, 15.31-, 11.41- and 3.14-fold decrease, respectively, compared to that of the ancestral strain. Compared to Group A hamsters, which received only 2 doses, the neutralizing antibody titers in Group D hamsters against VoCs increased proportionally with the additional third dose. Consequently, the third dose not only increased the neutralizing antibody titers against the Delta variant but also compensate the reduction in neutralizing antibody titers against the Beta and Gamma variants found in the W+W group.

We also explored the possibility of using the Beta variant version of S-2P adjuvanted with CpG 1018 and aluminum hydroxide as the third dose in Group E (W + W + B). As shown in Figure 2, at five weeks after the third injection of Group E hamsters, NT50 GMTs were 6643, 1889, 1034, 1306, and 3595 against the ancestral strain, Alpha, Beta, Gamma, and Delta variants, respectively. Compared to the neutralizing titer against the ancestral strain, that against the Alpha, Beta, Gamma, and Delta variants had 3.52-, 6.42-, 5.09- and 1.85-fold reduction, respectively. Two doses of ancestral S-2P combined with CpG and aluminum hydroxide followed by third dose of the adjuvanted Beta variant S-2P induced the best neutralization effect
against the ancestral stain and all VoCs when compared to the other groups, especially against the Delta
variant. The dosing regimens in Groups A to E resulted in 1.9- to 3.8-fold reduction of NT₅₀ GMT against the
Delta variant than that of the ancestral strain. However, the NT₅₀ titers against the ancestral strain were
different in each of the group. By far, the Beta variant S-2P would be most suitable for the third booster shot
before we develop the vaccine based on the Delta variant S-2P. Thus, we are able to induce a broad spectrum
of neutralizing antibodies against all VoCs by two doses of MVC-COV19 followed by the third dose of its
Beta version of S-2P.

Protection from the Delta variant challenge in hamsters immunized with two doses of MVC-COV1901
or combined with a third dose of MVC-COV1901 or its Beta variant version of S-2P.

At eight weeks after completion of the last immunization, hamsters were challenged with 10⁴ PFU of the
Delta variant and body weights were tracked up to six days post infection (d.p.i.). All the vaccinated groups
did not show weight loss up to six days after virus challenge, compared with the adjuvant control. The
protective effect was most significant at 6 d.p.i. in vaccinated groups, while the adjuvant only group
experienced significant weight loss (Figure 3).

Lung viral load was measured by viral RNA and 50% tissue culture infectious dose (TCID₅₀) assays.
Figure 4A shows that lung viral RNA in Groups A to E hamsters were lower than that of the adjuvant control,
and only that in Group E decreased significantly compared to adjuvant control. In contrast, the viral titers in
all of the vaccinated hamsters measured by TCID₅₀ were significantly lower than that of the adjuvant control
at 3 d.p.i. (Figure 4B). Note that viral load, especially viral titer measured by TCID₅₀ dropped noticeably at 6
d.p.i. in adjuvant control group due to hamsters’ natural immune response (Figure 4 B). Intriguingly, we have
found a moderately negative correlation (Spearman rₛ = -0.8227) between NT₅₀ titer against the Delta variant
from serum sampled five weeks after the final immunization and the number of viral genome at 3 d.p.i.
(Figure 5). The level of NT₅₀ titer after immunization could be predictive of the clearance of virus in the lungs
post viral challenge.
This study is our second hamster SARS-CoV-2 challenge study, whereas in the first study we have shown both low and high dose of S-2P were effective against live SARS-CoV-2 virus challenge in hamster; in this study we have extended our concept to variant-based booster dose and challenge with the Delta variant [16]. As the Delta variant has emerged to become the more infectious and the dominant strain in majority of the world, it has been chosen as our model virus for infection [21]. In the immunogenicity data, as expected, immunization with either two doses of ancestral S-2P or two doses of Beta variant S-2P could not confer broad protection against all strains tested. Ancestral S-2P was ineffective against the Beta and Gamma variants, whereas Beta variant S-2P induced higher neutralizing titers against only the Beta variant (Figure 2). Bivalent mixture of both ancestral and Beta variant S-2Ps had similar results with two doses of ancestral S-2P but with slightly increased immunogenicity against the Beta and Gamma variants. Three doses of ancestral S-2P was able to boost the titers against the Beta and Gamma to that of the bivalent vaccine and further increased the titers against the Alpha and Delta variants. This study reveals that using the Beta variant S-2P as the third dose induces the broad spectrum of increased NT50 against all variants as well as the ancestral strain. It is of interest to note the ratio between the NT50 of the ancestral strain and Alpha variant, and the ancestral strain and Delta variant remain relatively stable ranging from 3.52 to 3.79 for the former and 1.85 to 3.75 for the latter regardless of the regimen used when compared with the Beta or Gamma variants, which fluctuated variably (Figure 2).

All five regimens of vaccination protected hamsters from weight loss induced by the Delta variant infection (Figure 3). Notably, while group B produced poor antibody response against the Delta variant, this group of hamster did not experience any weight loss. Furthermore, the viral titers of Delta variant in Group B was significantly lower than that of the adjuvant control (Figure 4B), suggesting that the amount of anti-Delta antibodies and/or T cell immune responses induced by two doses of the Beta variant S-2P could reduce viral replication in the lungs and protect the hamsters from weight loss. T cell immunity also plays major role in concert with humoral immunity in vaccine- or infection-induced immunity against SARS-CoV-2 and clearance of virus [22-25]. Previous studies shows that memory T cell pool has been selected by prior
infection (or vaccination) can be activated upon encountering heterologous virus if cross-reactive epitopes are
shared between the two viruses [26]. The broad neutralizing ability of immunizing with ancestral strain and
Beta variant S-2Ps in succession could presumably have been induced by cross-reactivity of memory B cells
and T cells. This is similar to the concept of the original antigenic sin, in which previous exposure to a virus
can cause antibody response to preferably secrete antibodies against the first virus after exposure to a similar
virus strain due to shared epitopes [26]. Cross-reactivity of T cells have also been noted for rapid induction of
immunity following infection or immunization with SARS-CoV-2 [22]. Since neutralizing antibodies induced
by vaccines are polyclonal, they could also cross-react with shared epitopes between different variants.
Polyclonal antibodies induced by SARS-CoV-2 spike mRNA vaccine were profiled to consist of a mixture of
antibodies targeting the N-terminal domain (NTD) and the RBD and differ in their binding and neutralizing
abilities [27, 28]. After three doses of vaccination, the re-induction of immunity after virus challenge may
explain for the low viral RNA titer in hamster immunized with either of the three-dose regimens (Figure 4).
One interesting observation is the negative correlation between NT50 titer and viral RNA of the Delta variant
(Figure 5). However, the TCID50 in all groups were very low and almost undetectable in most of the cases
(Figure 4). This may due to the sensitivity of the TCID50 assay itself or the viral RNA assay may be detecting
fragments of viral RNA from dead viruses as opposed to live replicating viruses. In future studies,
subgenomic RNA detection should also be used to detect replicating viruses to corroborate with the TCID50
results. The establishment of correlates of protection using the relationship between NT50 titer and viral RNA
in a given hamster challenge model will facilitate expedited evaluation of vaccine combination in future
development process.

One limitation of this study is that we have not tested the vaccine’s protection in vivo with other VoCs,
so the vaccine efficacy against other VoCs is inferred from the neutralizing antibody titers. The natural course
of infection among the hamsters includes convalescent state, so the model does not allow for evaluating
mortality or severe disease as endpoints. T-cell functions were not evaluated, limiting the assessment of role
of cellular immunity in the role of protection. Two recent studies investigated the effects of booster dose of
ChAdOx1 and mRNA-1273 [9, 29]. Administration of a third dose using Beta variant version of mRNA-1273
(mRNA-1273.351) after two doses of mRNA-1273 had lower adverse events and increased immunogenicity against the Beta variant than three doses of mRNA-1273; while administration of either mRNA-1273 or mRNA1273-351 as third dose exponentially boosted immunogenicity against all variants tested compared to two doses of mRNA1273 [9]. While in ChAdOx1, the third dose induced generally less adverse events than that of first or second dose, and third dose boosted neutralization titers against the Beta and Delta variants as well as gamma-interferon levels [29]. These findings appear to corroborate our results that a third dose of vaccination could boost immune response against the virus and its variants.

As an extension of phase 1 clinical trial of MVC-COV1901, the participants were administered with a booster vaccination of the original S-2P at 180 days after the second immunization to investigate the clinical effect of the booster shot (NCT04487210). At the time of administering the booster dose in the phase 1 extension trial, only the original S-2P based on the ancestral strain was available; however, based on the result of group D in this study, we expect to see boosting immunogenicity against the variants compared to the current two-dose regimen. An ideal effect of an antigen construct of a booster vaccine after the primary series should be able to increase protection against the prevailing VoC, such as the Delta strain, and at the same time, render adequate protection against vaccine escape strains, such as the Beta stain. The third shot (second booster) using a Beta strain vaccine construct in our hamster model demonstrated wider breath of cross-reactivity against VoCs and similar protection against the Delta strain compared with the third shot using the ancestral strain vaccine. The booster and challenge study results in hamsters and the phase 1 extension studies booster results in humans provide us with potential strategies against the VoCs in the future by administering a Beta variant S-2P as the booster shot. These results support our further development plan to test the Beta variant S-2P vaccine as a booster after the primary series of MVC-COV1901 vaccine in clinical settings.

**Methods**

**Animals and ethical 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. At 3 weeks after the final immunization, the animals were transferred to Academia Sinica, Taiwan, for 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 (IACUC) 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 Figure 1. The hamsters were split into the following six groups with n = 10 for each group (Table 1):

<table>
<thead>
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<th>Groups</th>
<th>First immunization</th>
<th>Second immunization</th>
<th>Third immunization</th>
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<tbody>
<tr>
<td>A: W + W</td>
<td>Ancestral strain (1 μg)</td>
<td>Ancestral strain (1 μg)</td>
<td>-</td>
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<tr>
<td>B: B + B</td>
<td>Beta variant (1 μg)</td>
<td>Beta variant (1 μg)</td>
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<td>C: (W + B) + (W + B)</td>
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<td>Ancestral strain (1 μg)</td>
<td>Ancestral strain (1 μg)</td>
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<tr>
<td>E: W + W + B</td>
<td>Ancestral strain (1 μg)</td>
<td>Ancestral strain (1 μg)</td>
<td>Beta variant (1 μg)</td>
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<td>F: Adjuvant control</td>
<td>CpG 1018 (150 μg) and aluminum hydroxide (75 μg)</td>
<td>CpG 1018 (150 μg) and aluminum hydroxide (75 μg)</td>
<td>CpG 1018 (150 μg) and aluminum hydroxide (75 μg)</td>
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Hamsters in group A were vaccinated on days 22 and 43 with 1 μg of S-2P protein derived from the ancestral strain. Hamsters in group B were vaccinated on days 22 and 43 with 1 μg of S-2P protein derived from Beta variant. Hamsters in group C were vaccinated on days 22 and 43 with a mixture of the ancestral strain (0.5 μg) and Beta variant (0.5 μg) of S-2P protein (bivalent vaccine). Hamsters in group D were vaccinated on days 1, 22, and 43 with 1 μg of S-2P protein derived from the ancestral strain. Hamsters in group E were vaccinated on days 1 and 22 with 1 μg of S-2P protein derived from the ancestral strain, and on day 43 with 1 μg of S-2P protein derived from the Beta variant. Hamsters in group F served as an adjuvant control and were vaccinated with only 150 μg of CpG 1018 and 75 μg of aluminum hydroxide (alum) on days 1, 22 and 43. All immunization with S-2P were adjuvanted with 150 μg of CpG 1018 and 75 μg of alum.

Serum samples were collected five weeks after the final immunization and immunogenicity was determined by neutralization assay with SARS-CoV-2 virus and the variants. Approximately three weeks after the serum sampling (53 days after the final immunization), hamsters were challenged with the SARS-CoV-2 Delta variant (hCoV-19/Taiwan/1144) and then sacrificed at 3 d.p.i. (n = 5 per group) or 6 d.p.i. (n = 5 per group) for analyses of lung viral loads, lung TCID₅₀. Body weight of individual hamsters were tracked daily up to the time of sacrifice.

**Live SARS-CoV-2 neutralization assay**

SARS-CoV-2 virus and variants used in the assay consisted of the follow obtained from the Taiwan CDC: Ancestral (Wuhan) strain (hCoV-19/Taiwan/4/2020, GISAID EPI_ISL_41192), Alpha (hCoV-19/Taiwan/792, GISAID EPI_ISL_1381386), Beta (hCoV-19/Taiwan/1013), Gamma (hCoV-19/Taiwan/906), and Delta (hCoV-19/Taiwan/1144). Live virus neutralization assay were performed as described previously [18].

**Viral RNA quantification and cell culture infectious assay (TCID₅₀)**

Quantification of lung viral load by real-time PCR and TCID₅₀ assay were performed as previously reported [16].
Statistical analysis

The analysis package in Prism 6.01 (GraphPad) was used for statistical analysis. Spearman’s rank correlation coefficient and linear regression were calculated for Figure 5. Kruskal-Wallis with corrected Dunn’s multiple comparisons test and two-way ANOVA with Dunnett test for multiple comparison were used to calculate significance in Figures 2 to 4 where appropriate. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001

Acknowledgements

We would like to thank the team members at TFBS Bioscience Incorporation for hamster housing and immunization process, and the Biosafety Level 3 Facility of Academia Sinica, Taiwan, for providing environment to handling and performing SARS-CoV-2 hamster challenge and live SARS-CoV-2 neutralization assay. We also thank John D Campbell and Paula Traquina of Dynavax Technologies for reviewing and providing helpful inputs toward the manuscript.

Author Contributions

T.-Y. K., C.-C. W., and W.-H. T. produced the ancestral and Beta variant versions of S-2P antigens 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., Y.-S. C., and L. T.-C. L. drafted the manuscript. All authors reviewed and approved of the final version of the manuscript.

Competing Interests


19. Available at:


29. Flaxman A, Marchevsky NG, Jenkin D, et al. Reactogenicity and immunogenicity after a late second
dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials

(COV001 and COV002). The Lancet. 2021 Sep 1.

Figures

Figure 1. Study design of the hamster challenge study.
Hamsters (N=10 per group) were immunized twice (groups A, B, and C) or three times (groups D, E, and F) at three weeks apart and serum samples were taken for immunogenicity assays five weeks after the final immunization. Eight weeks after the final immunization, hamsters were challenged with $10^4$ PFU of SARS-CoV-2 Delta variant. The animals were euthanized on the third or sixth day after infection for necropsy and tissue sampling to determine viral load. Body weight of individual hamster were tracked daily up to the time of sacrifice.

**Figure 2. Neutralizing antibody titers with live SARS-CoV-2 neutralization assay in hamsters five weeks after the final immunization.** Hamsters were immunized as in Figure 1. 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 ancestral strain and Alpha, Beta, Gamma, and Delta variants. Results are shown as bars indicating the NT$_{50}$ GMT with individual values displayed as symbols and error bars showing the 95% confidence intervals. Average fold reduction in GMT of variants against the ancestral strain were calculated and shown above brackets above the corresponding bars. W: ancestral (Wuhan) strain S-2P; B: Beta variant S-2P; W + B: bivalent mixture of ancestral and Beta variant S-2Ps. Statistical significance was calculated with Kruskal-Wallis test with corrected Dunn’s multiple comparisons test.
Figure 3. Change in body weight in hamsters after infection with SARS-CoV-2 Delta variant.

Hamsters were challenged with SARS-CoV-2 Delta variant eight weeks after the final immunization. The body weights of individual hamsters were tracked daily up to the time of euthanizing at six days post infection (n = 5/group). Results are shown as percent of weight relative to the day of challenge (day 0). Statistical significance was calculated with two-way ANOVA with Dunnett multiple comparison test with adjuvant only as control.

Figure 4. Viral load in hamsters three or six days post infection with SARS-CoV-2 Delta variant.

The hamsters were euthanized at three or six days (n = 5/group) after infection and lung tissue samples were collected for viral load determination by A. quantitative PCR of viral genome RNA, and B. TCID$_{50}$ assay for virus titer. Results are presented as geometric means with individual values represented by symbols and error bars representing 95% confidence interval. Statistical significance was calculated with Kruskal-Wallis test with corrected Dunn’s multiple comparisons test.
Figure 5. Correlation between SARS-CoV-2 viral genome copy numbers and NT$_{50}$ titers against the Delta variant. Values of viral genome copy numbers 3 days post infection and NT50 titers against the Delta variant five days after the final immunization were tabulated (n = 29). Spearman’s rank correlation coefficient and linear regression were calculated with dotted bands and shaded area representing the 95% confidence bands of the linear regression line.
Groups A, B, and C (n=10/group)

**Prime** Day 1
**Boost** Day 22  3 weeks
**Bleed** Day 57  5 weeks
**Challenge** Day 75
SARS-CoV-2 Delta variant
1 x 10^4 PFU, 50 μl intranasally
**Sacrifice** 3 dpi (n=5/group) or 6 dpi (n=5/group)  3 to 6 days
Day 78 or 81

Neutralizing antibody titer

Viral load: 3 and 6 dpi
Body weight: 0 to 6 dpi

Groups D, E, and F (n=10/group)

**Prime** Day 1
**Boost** Day 22  3 weeks
**2nd Boost** Day 43  3 weeks
**Bleed** Day 78  5 weeks
**Challenge** Day 96
SARS-CoV-2 Delta variant
1 x 10^4 PFU, 50 μl intranasally
**Sacrifice** 3 dpi (n=5/group) or 6 dpi (n=5/group)  3 to 6 days
Day 99 or 102

Neutralizing antibody titer

Viral load: 3 and 6 dpi
Body weight: 0 to 6 dpi
Average NT fold reduction over ancestral strain

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NT₅₀ (GMT+/- 95%CI)

- A: W + W
- B: B + B
- C: (W + B) + (W + B)
- D: W + W + W
- E: W + W + B
- F: Adjuvant control

Legend:
- Blue: Ancestral
- Red: Alpha
- Green: Beta
- Pink: Gamma
- Orange: Delta

Significance:
- ***: p < 0.001
- **: p < 0.01
- *: p < 0.05
Spearman $r_s = -0.8227$
$P < 0.0001$

$y = -0.884x + 13.18$
$R^2 = 0.7226$