PLOS ONE

RESEARCH ARTICLE

Humoral immunogenicity of a Coronavirus Disease 2019 (COVID-19) DNA vaccine in rhesus macaques (*Macaca mulatta*) delivered using needle-free jet injection

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Abstract

A SARS-CoV-2 DNA vaccine targeting the spike protein and delivered by jet injection, nCOV-S(JET), previously shown to protect wild-type and immunosuppressed Syrian hamsters (*Mesocricetus auratus*), was evaluated via two needle-free delivery methods in rhesus macaques (*Macaca mulatta*). The methods included intramuscular delivery of 2 mg per vaccination with the PharmaJet Stratis device and intradermal delivery of 0.4 mg per vaccination with the PharmaJet Tropis device. We hypothesized that the nCOV-S(JET) vaccine would mount detectable neutralizing antibody responses when delivered by needle-free jet injection by either the intradermal or intramuscular route. When delivered intramuscularly, the vaccines elicited neutralizing and variant (Beta, Gamma, and Delta) cross-neutralizing antibodies against SARS-CoV-2 in all six animals after three vaccinations. The neutralizing response to Omicron was lower with only 4 of 6 animals responding. When delivered at a lower dose by the intradermal route, strong neutralizing antibody responses were only detected in two of six animals. This study confirms that a vaccine previously shown to protect in a hamster model can elicit neutralizing and cross-neutralizing antibodies against SARS-CoV-2 in nonhuman primates. We posit that nCOV-S(JET) has the potential for use as booster vaccine in heterologous vaccination strategies against COVID-19.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiologic agent of Coronavirus Disease 2019 (COVID-19), was declared a pandemic by the World Health Organization (WHO) on March 11th, 2020, after its initial discovery in Wuhan, China, in December 2019 [1]. Two years later, nine vaccines have been approved for emergency or full use by at least one stringent regulatory authority and 147 more are in clinical trials [2–4]. Despite a

global effort to expeditiously produce safe and efficacious vaccines, the virus has evolved and vaccines targeting new variants of concern will be needed for the foreseeable future. Two of the three COVID-19 vaccines approved for full use in the United States utilize an mRNA platform (BNT162b2, Pfizer-BioNTech; mRNA-1273, Moderna) and have strict cold chain requirements, which poses significant logistical challenges. Platforms such as DNA vaccines which have a longer shelf life and simpler storage conditions [5] could play a role in future vaccine strategies. Several COVID-19 DNA vaccines are currently in clinical trials [4]. The multi wave nature of the COVID-19 pandemic has made it evident that prolonged periods of insufficient vaccination in a population give the virus ample time to mutate and potentially evade vaccination efforts. Thus, it may become necessary to rapidly develop variant-targeted vaccines to boost both vaccine-generated and natural immunity against the ancestral virus.

Previously we described the development and testing of a needle-free, jet-injected, spike-based SARS-CoV-2 DNA vaccine, nCoV-S(JET), in immunocompetent and immunosuppressed Syrian hamster (*Mesocricetus auratus*) models [6]. In that study, neutralizing antibodies levels reached relatively high levels after two vaccinations and significantly reduced disease as measured by body weight loss, lung pathology and virus burden. We were interested in determining if the vaccine would produce similar immunogenicity in a nonhuman primate (NHP) model. We hypothesized that all rhesus macaques (*Macaca mulatta*) would produce detectable neutralizing antibody responses when vaccinated intramuscularly (IM) or intradermally (ID) with nCoV-S(JET).

Herein, we describe the testing of nCoV-S(JET) in rhesus macaques using PharmaJet Stratis and Tropis devices. The Stratis and Tropis devices deliver a needle-free jet of liquid intramuscularly or intradermally, respectively. Our primary endpoints for this immunogenicity study were neutralizing antibodies measured by live virus plaque reduction neutralization tests (PRNT) and pseudovirion neutralization assays (PsVNA).

Materials and methods

Virus stocks

A third passage SARS-CoV-2 strain WA-1/2020 viral stock was obtained from the CDC and is from a human non-fatal case isolated in January 2020. A master stock of virus was propagated as described previously [7]. A master stock of virus of NR-54009 (Beta) was also propagated in a similar manner. NR-55674 (Delta) and NR-54984(Gamma) were obtained and used directly from the BEI, American Tissue Culture Collection. All virus work was conducted in BSL-3 containment at USAMRIID.

Nonhuman primate experimental design

Twelve experimentally-naïve rhesus macaques aged 8 to 15 years of Chinese origin and weighing 5 to 16 kg were utilized for this study. Vaccination groups were randomized and composed of 3 males and 3 females each. Animals underwent a physical examination and bloodwork to confirm lack of underlying disease conditions prior to enrollment on the study. When socially-compatible partners were available, animals were housed in pairs. In addition to primary feed and water, environmental enrichment was provided in the form of toys, treats, and fresh fruits and vegetables Animals were sedated with ketamine (Ketamine HCl, 100 mg mL-1, Dechra Veterinary Products, Overland Park, KS; 10 mg kg-1) for all procedures.

Animals received either 0.4 mg of SARS-CoV-2 DNA vaccine, nCOV-S(JET), intra-dermally via PharmaJet Tropis device or 2.0 mg intramuscularly via PharmaJet Stratis device. The immunization volume was split in half and administered on the right and left lower axilla or triceps muscles respectively. All animals were vaccinated on days 0, 21 and 42 and serum and

whole blood were collected on days 0, 21, 35, 63, and 168 with blood collection preceding vaccination on days where both procedures occurred. Animals were observed daily for clinical and behavioral abnormalities for the duration of the study. There was no challenge with SARS-CoV-2 virus in this study, so the animals did not develop disease. At the end of the study the animals were returned to the colony.

Research was conducted under an IACUC approved protocol at USAMRIID (USDA Registration Number 51-F-00211728 & OLAW Assurance Number A3473-01) in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the AAALAC, International and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, *National Research Council*, 2011.

Plaque Reduction Neutralization Test (PRNT)

The PRNTs were conducted as described previously [8]. An equal volume of complete media (MEM containing 10% heat inactivated FBS, 2% Pen/Strep, 100X NEAA, 1% HEPES, 0.1% Gentamycin, and 0.2% Fungizone®) containing SARS-CoV-2 was combined with 2-fold serial dilutions of cMEM containing antibody and incubated at 37˚C in a 5% CO_2 incubator for 1 hour. Afterwards, the combined virus/antibody mixtures were then added to 6-well plates (180 μl/well) containing 3-day old, ATCC Vero-76 confluent cell monolayers and allowed to adsorb for 1 hour in a 37˚C, 5% CO_2 incubator. Three milliliters of agarose overlay (0.6% Sea-Kem® ME agarose, 2X EBME with HEPES, 10% heat-inactivated FBS, 100X NEAA, 4% GlutaMAXTM, 2% Pen/Strep, 0.1% Gentamycin and 0.2% Fungi-zone®) per well was then added and allowed to solidify at room temperature. The plates were placed in a 37˚C, 5% CO_2 incubator for 2–3 days (variant dependent) and then 2 mL per well of agarose overlay stain (0.6% SeaKem® ME agarose, 2X EBME with HEPES, 5% heat-inactivated FBS, 2% Pen/Strep, 0.1% Gentamycin, 0.2% Fungizone®, 5% Neutral Red) was added. Once plaques could be visualized (one to two days post-staining), plates were removed from the incubator and counted on a light box. PRNT50 titers were determined and are defined as the reciprocal of the highest dilution that results in a 50% reduction in the number of plaques relative to the average number of plaques visualized in the cMEM alone (no antibody) wells.

Plasmids used for vaccinations

The full-length S gene open reading frame, preceded at the N-terminus by the Kozak sequence (ggcacc), was human codon usage-optimized and synthesized by Genewiz (South Plainfield, NJ) and cloned into the Notl-BgIII site of the DNA vaccine vector pWRG for the pWRG/nCoV-S(opt) [6]. The SARS-nCoV-2 S sequence used was the Wuhan coronavirus 2019 nCoV S gene open reading frame (Genebank accession QHD43416). The plasmids for use in vaccinations were produced commercially and diluted in PBS to 2 mg/mL (Aldevron, Fargo, ND). The pWRG/nCoV-S(opt) plasmid is also called nCOV-S(JET) when combined with jet injection.

Plasmids used for pseudovirion production

A second plasmid for the PsVNA was constructed by deletion of 21 amino acids from the COOH terminus of the full-length plasmid, pWRG/CoV-S(opt) Δ21 for better in-corporation into pseudovirions [6]. The truncated form was generated using the protocol of 98˚C for 2 min, followed by 25 cycles of, "98˚C for 10 s, 65˚C for 10 s, 72˚C for 2 min" and 72˚C for 10min. The forward and reverse primer sequences are: Fwd- 5′-CCGGCCGCGGCCGCGCCACCATGTTTGTGTTTCTGGTCCTCCTC-3′, Rev-5′-

GCTGTTGTAGCTGCGGAAGCTAGTAGTAGGCTAGCAGATCTGCGC-3′. The PCR fragment was gel purified and cloned into the NotI-BgIII site of the DNA vaccine vector pWRG. The Beta (CodexDNA, San Diego, CA) and Omicron (TWIST, South San Francisco, CA) truncated constructs were synthesized directly into the pWRG backbone. For the Delta variant a full-length plasmid was acquired from GenScript (Piscataway, NJ) and then truncated (last 21 amino acids) by in-house PCR and cloned into pWRG using NotI/BamHI. The open reading frame amino acid sequence used for the variants had the following mutations as compared to Wuhan: Beta (D80A, D215G, R246I, K417N, E484K, N501Y, A701V), Delta (G142D, Δ156–157, R158G, L452R, T478K, P681R, D950N), and Omicron (A67V, Δ69–70, T95I, Δ142–144, Y145D, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F). Expression of the spike proteins from pWRG/Delta, pWRG/Beta, and pWRG/OM was confirmed by transfection of 293T cells followed by immuno-fluorescence antibody test (IFAT) using heat inactivated (56˚C 30 min) human convalescent plasma NRS-53265 (ATCC, Manassas, VA) and compared to empty vector.

Pseudovirion production

Pseudovirions were produced using the pWRG/CoV-S(opt)Δ21, pWRG/CoV-S(Beta)Δ21, pWRG/CoV-S(Delta)Δ21 and pWRG/CoV-S(OM) Δ21 plasmids described above using previously described methods [6]. Briefly, HEK293T cells were transfected with the aforementioned plasmids and after 18 hrs cells were infected with VSVΔG*rLuc. After 3 days at 32˚C pseudovirions were concentrated by PEG precipitated and then resuspended in TNE buffer. Pseudovirions were stored at -70˚C.

Pseudovirion Neutralization Assays (PsVNA)

The PsVNA used to detect neutralizing antibodies in sera utilized a nonreplicating vesicular stomatitis (VSV)-based luciferase expressing system [9] with two modifications as previously described [6]. The modifications were (1) no supplemental human complement was added to heat inactivated sera, (2) to reduce any low level residual VSV activity a neutralizing monoclonal antibody was added. A regression model (four parameter logistic (4PL) commonly used for bioassays was used to interpolate the PsVNA50 and PsVNA80 titers from which GMTs were calculated.

MAGPIX multiplex immunoassay

MAGPIX using the SARS-CoV 2 spike, S1, RBD, and NP were conducted as previously described [10]. This raw data was converted to a fold change in signal over the pre-bleed by dividing the signal from the study timepoint samples by the prebleed sample. Any sample with a fold change greater than four was considered positive.

Results

Twelve rhesus macaques (*Macaca mulatta)* were vaccinated with nCOV-S(JET) as out-lined in **Table 1**. Sera were collected on days 0, 21, 35, 63, and 168 and evaluated for neutralizing antibodies via plaque reduction neutralization (PRNT) and pseudovirion neutralization assays (PsVNA) as well as for binding antibodies via MAGPIX multiplex as-say. Animals were observed daily for signs of adverse reaction, including erythema or other abnormalities at the site of vaccination, changes to food or enrichment consumption, behavioral changes, or

Table 1. Experimental design. Groups of 6 rhesus macaques each were vaccinated with the nCOV-S(JET) DNA vaccine and sera were collected to evaluate neutralizing and binding antibodies.

Group	PharmaJet Device	Total Dose per Vaccination	Route	Site	Procedure Schedule (Days)	
					Vaccination	Blood Collection
ID-DSJI	Tropis	0.4 mg	ID	Below axilla	0, 21, 42	0, 21, 35, 63, 168
IM-DSJI	Stratis	2.0 mg	IM	Triceps		

https://doi.org/10.1371/journal.pone.0275082.t001

change in clinical status. No evidence of adverse reaction following vaccination were noted in any animals during the study.

Neutralizing antibodies

Pre-vaccination, all animals were negative for neutralizing antibodies by both PRNT and PsVNA (**Figs 1 and 2**). After a single vaccination, two animals in the IM-DSJI group and no animals in the ID-DSJI were positive on at least one of the assays. After two vaccinations, 5 of 6 (83.3%) of the IM-DSJI vaccinated animals developed neutralizing antibodies as compared to 1 of 6 (16.67%) of ID-DSJI vaccinated animals. After 3 vaccinations, 100% of the IM-DSJI group developed neutralizing antibodies as compared to only 33% (2 of 6) of the ID-DSJI group. An additional blood collection was performed on Day 168 to look at durability of the response (**Fig 2**). All but one of the IM-DSJI were still positive in the WA-1 PsVNA, whereas only 50% were still positive by PRNT. The single ID-DSJI vaccinated NHP that was positive by PRNT on Day 63 was still positive on Day 168. That animal, along with two other animals in that group, were positive by WA-1 PsVNA on Day 168. Those two animals had previously been negative by PsVNA on Day 63. Thus, the overall neutralizing antibody positivity rate for the ID-DSJI device was 4 of 6 (66.7%), albeit with low titers.

Binding antibodies

Magpix assays were performed on sera collected through Day 63 and plotted (**Fig 1**). The assay measured IgG responses against the full spike, S1 domain, receptor binding domain (RBD), and nucleocapsid (N) protein. Predictably, the N response was negative in all animals at all timepoints. After one IM-DSJI vaccination, the same two animals positive for neutralizing antibodies were also positive for full spike and S1. One of those two animals (#4) also had a high anti-RBD response. A third animal (#5) had detectable anti-RBD antibody but was negative for other proteins. After two vaccinations, all the IM-DSJI animals had at least low levels of binding antibodies against at least two target proteins. Only one animal in the ID-DSJI group had positive binding anti-bodies after two vaccinations (animal #7). After three vaccinations, all the animals in the IM-DSJI group had improved binding antibody responses and 5 of 6 animals in the ID-DSJI group had detectable binding antibody against at least one target. Animal #10 had almost undetectable responses. The binding data confirmed what was observed in the neutralizing antibody assays: the IM-DSJI device delivering 2 mg was more immunogenic than the ID-DSJI device delivering 5-fold less DNA.

Cross-neutralizing activity against variants of concern (VOC)

Peak Day 63 sera were evaluated for cross-neutralization activity against Beta, Delta, Gamma and Omicron SARS-CoV-2 VOC. Delta and Gamma variants were evaluated by PRNT and Beta, Delta, and Omicron variants by PsVNA. 100% of IM-DSJI vaccinated animals showed cross-neutralization activity against all VOC except the Omicron with the highest reactivity shown against Delta. The response to Omicron was markedly reduced with only 4 of 6 animals positive, and
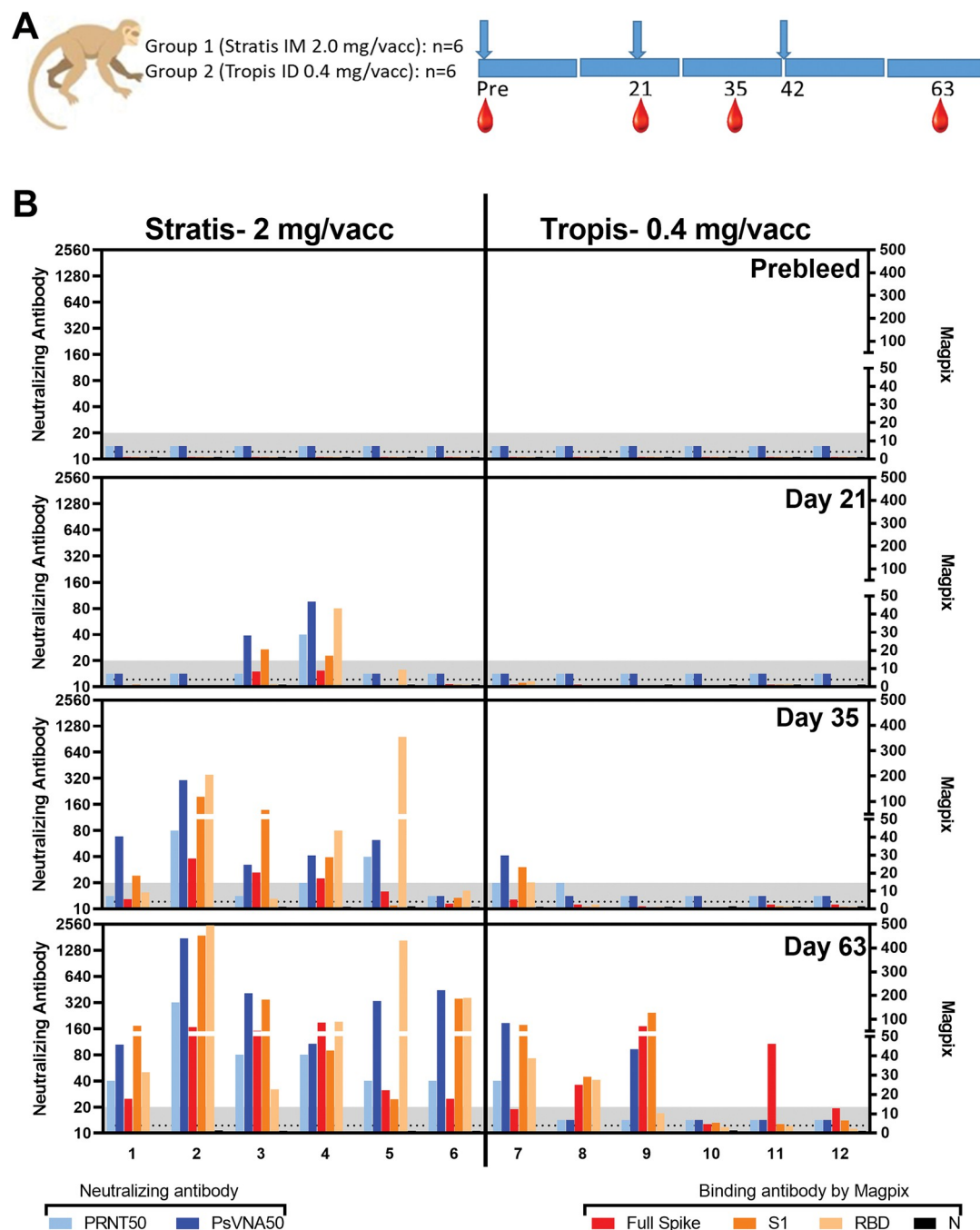
Fig 1. Neutralizing and binding antibody responses. PRNT50, PsVNA50, and Magpix titers from sera collected at various timepoints. **A)** Design. (blue arrows = vaccine dosing; red drops = blood collection time points). **B)** Neutralizing and binding antibody values at the indicated timepoints. The neutralizing antibody titers are shown on the left y-axis and the Magpix fold-change over prebleed on the right y-axis. The numbers on the x-axis represent animal identifications. There are 5 bars for each animal at each timepont. Bar colors and their respective assays are defined at the bottom of the figure. Neutralization assay lower limits are shown as gray shaded area and the 4-fold limit for positive Magpix is shown as dotted line.

only one of those with a PsVNA >100. All ID-DSJI vaccinated animals showed detectable cross-neutralization activity against at least one variant; however none of the animals showed cross-neutralization activity against all VOC. Cross-neutralization data are shown in **Fig 3**.
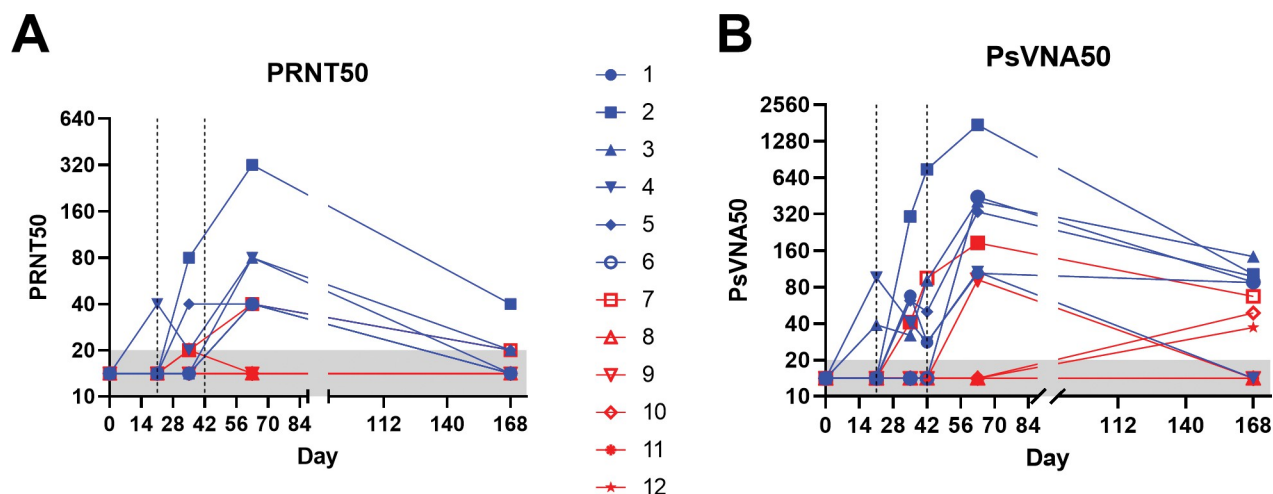
Fig 2. Kinetics of neutralizing antibody responses of individual animals. A) PRNT50 and **B)** PsVNA50 titers from sera collected at various timepoints. Vertical dashed lines indicate vaccination timepoints. Blue symbols/lines represent IM-DSJI-vaccinated animals. Red symbols/lines represent ID-DSJI-vaccinated animals. Assay lower limits are shown as gray shaded area.

Discussion

This report describes the immunogenicity of the SARs-CoV-2 DNA vaccine nCOV-S(JET) by jet injection in a rhesus macaque model. Our goal was to confirm that the nCOVS(JET) DNA could elicit a detectable neutralizing antibody response and to determine the relative potency
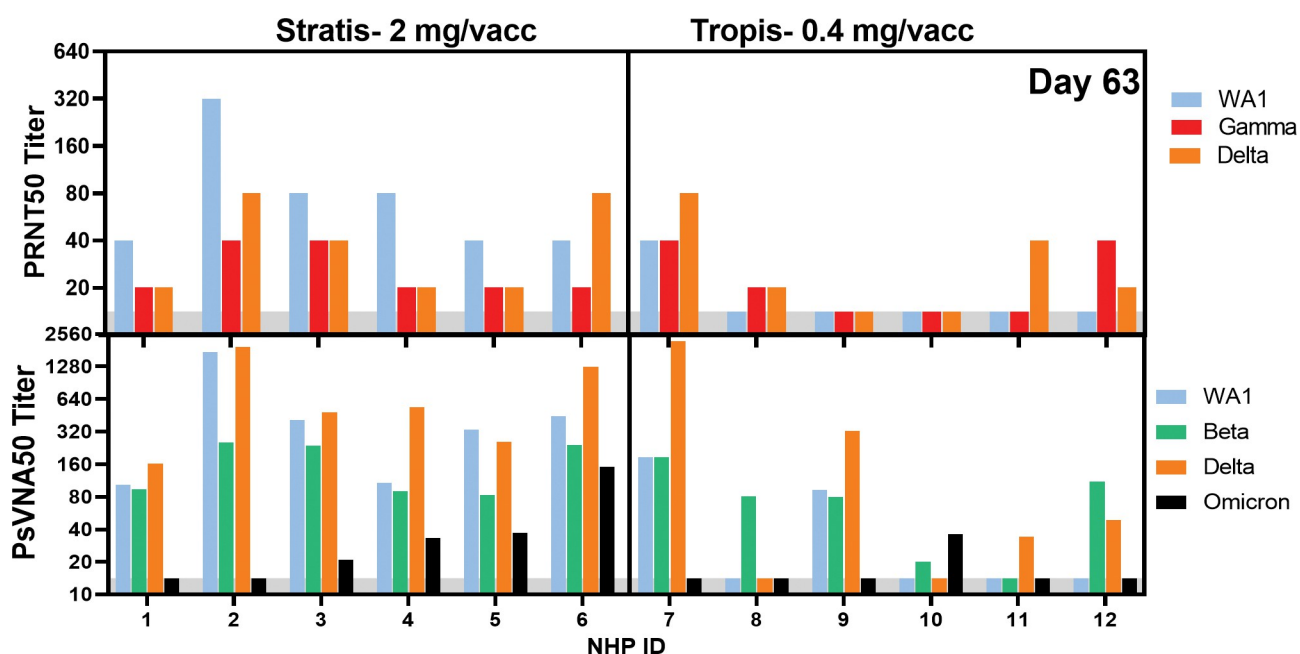

Fig 3. Cross-neutralizing antibody titers against SARS-CoV-2 VOCs from day 63 sera. The top panel reports the PRNT50 titers, and the bottom panel the PsVNA50 titers for each animal. Animal identification (ID) are shown on x-axis. Animals 1–6 were vaccinated using the Stratis intramuscular jet injection device and animals 7–12 were vaccinated with 0.4 mg using the Tropis intradermal jet injection device. For the PRNT, there are three bars for each animal representing the titers against the vaccine strain (WA1), the Gamma variant, or the Beta variant of concern (VOC). For the PsVNA, there are four bars for each animal representing the titers against the vaccine-based pseudovirion WA1, or pseudovirions for the Beta, Delta, and Gamma VOC. Assay lower limits are shown as gray shaded area.

of an IM versus ID delivery of the vaccine by jet injection. All IM-DSJI NHPs successfully mounted neutralizing antibodies after 3 vaccinations as compared to only 4 of 6 ID-DSJI NHPs. Additionally, neutralizing antibody responses occurred more quickly in the IM-DSJI NHPs, with 100% mounting responses by Day 63 as compared to only 2 of 6 ID-DSJI NHPs. One limitation of this study is that we focused on the humoral immune response and did not conduct a comprehensive assessment of the vaccine-mediated immune response including T cell responses. An additional limitation was that we did not deliver the same dose of DNA by the two different routes. Our rationale for delivering different doses was that we delivered the highest dose possible using a standard 2 mg/mL concentration of vaccine drug product. For the IM Stratis device this was 2 mg delivered as two 0.5 mL injections; and for the ID Tropis device this was 0.4 mg delivered as two 0.1 mL injections. Obviously, these doses could be increased by starting with more concentrated DNA. Nevertheless, we did determine that the doses used were capable of eliciting neutralizing antibodies in NHPs as measured by both PsVNA and PRNT.

Neutralizing antibody titers failed to rise in the rhesus macaque model as quickly as was seen in Syrian hamsters, with the macaques requiring an additional (second) boost to reach similar antibody titers [6]. The geometric mean titer (GMT) PsVNA50 in hamsters after two vaccinations was approximately 640 whereas in this experiment in NHPs the GMT PsVNA50 was 58 after two vaccinations and 326 after three vaccinations. Similarly, GMT PRNT50 in hamster after two vaccinations was approximately 640 whereas in this experiment in NHPs the GMT PRNT50 was 24 after two vaccinations and 71 after three vaccinations. Using the same PRNT assay we found that macaques infected with WA-1 virus developed PRNT80 titers between 160 and 2560 18 days after exposure [10]. The corresponding PRNT50 titers in that study were between 640 and 5120 (data not shown). It is possible that the NHPs received an inadequate dose as compared to the hamsters when juxtaposed on a per weight basis. Hamsters received a total of 0.4 mg nCOV-S(JET) intramuscularly over three vaccinations, as compared to the 6.0 mg received intramuscularly in the NHPs. While our NHP dosing is similar to other vaccines utilizing DNA platforms, the dose used in our small animal model was substantially higher [11–13]. Since our previous work in hamsters only explored vaccine efficacy via the intramuscular route we cannot truly compare potential discrepancies in the dosing per weight, but DNA vaccines historically have demonstrated higher immunogenicity in rodents relative to primates, and the highest immunogenicity when administered intramuscularly as compared to other routes [14].

In a highly relevant study, Lassauniere *et al*. vaccinated rhesus macaques with a SARS-CoV-2 DNA vaccine delivered using Tropis and then challenged with the WA-1 variant and found the vaccine protected [15]. In that study the dose of plasmid delivered by Tropis was 5x higher than the doses we used (*i.e.*, 4 administrations of 0.5 mg per vaccination vs 2 administrations of 0.2 mg per vaccination). The PRNT50 titers in the Lassaumiere study after 3 vaccinations ranged from 30–125 whereas in our study the PRNT50 in the Stratis group ranged from 40 to 320. Although we did not conduct a challenge experiment, the level of neutralizing antibodies is similar the level produced in Lassaumiere study, where protection was achieved [15]. The levels of neutralizing antibodies are also similar to those produced by 2 doses of plasmid DNA delivered by ID-EP [16] or 2 dose of 5 mg of plasmid DNA delivered by needle and syringe [17]. In both of those studies the PRNT50 titers ranges from 20 to 540 and the NHP exposed to SARS2 virus had reduced viral loads in their lungs [16,17].

A spike-based DNA vaccine termed ZyCoV-D delivered three times to rabbits intradermally using the Tropis device elicited neutralizing antibody titer of 108 in a microneutralization test [13]. The vaccine administered at a dose of 2 mg/vaccination three times using Tropis to humans in a phase 1 clinical resulted in a neutralizing antibody GMT of 39.17 [18]. Thus,

the neutralizing antibody response we observed in NHP vaccinated with 2 mg by the IM-DSJI route were comparable to titers that were protective in NHPs, and similar to the titers elicited in rabbits of a vaccine that has been approved for use in humans in India.

The nCOV-S(JET) vaccine delivered using the IM Stratis device exhibited impressive VOC cross-neutralizing activity, especially when measured by the PsVNA. the study reported herein, all of the animals had PsVNA50 titers against WA-1, Beta, and Delta of at least 80 and were as high as 1280. Interestingly, the responses against Delta appeared to be as high, or higher, than against WA-1. The lack of a diminished response against Delta was also seen in other studies. [15]. Cross-neutralizing activity against Omicron was detectable with the IM Stratis device, but was markedly lower than other VOC's responses. Cross-neutralization against VOC with the lowest response against Omicron has been reported for a DNA vaccine delivered by electroporation in NHP, so this is not totally unexpected [17]. In The PRNT50 were less impressive, but all of the IM-DSJI NHPs had PRNT50 titers of at least 20 and as high as 80 against the Gamma and Delta VOC. Unsurprisingly, the low dose of DNA delivered by ID Tropis device had lower cross-neutralizing responses. Only two animals (#7 and #9) had detectable cross-neutralizing antibodies against all VOC tested, excluding Omicron, (by PsVNA) and only one of those animals was also positive against all VOC tested by PRNT (animal #7). These two animals also had the most robust binding response as measured by Magpix indicating high levels of binding antibodies is predictive of a potent neutralizing and cross-neutralizing response. There were only two VOC run in both the PRNT and PsVNA, WA1 and Delta. In general, the assays results were very similar although there was a bias for higher titers in the PsVNA. For example, NHP ID #2 had the highest WA-1 titer in both assays. Correlation analysis for the WA-1 and Delta PRNT vs PsVNA both yielded Pearson r values greater than 0.9 indicating a strong correlation.

At the doses used in this study, two boosts were necessary to produce responses that would likely be beneficial to the vaccine recipient (i.e., neutralizing antibody responses). This number of vaccinations is not well suited for rapid, large scale vaccination campaigns, but may be most appropriate for heterologous boosting strategies. Heterologous boosting, wherein the booster vaccine is of a different platform than that used to complete the primary vaccination series, allows for increased flexibility, a significant advantage in the current landscape of vaccine shortage, and has the potential for reduced reactogenicity and increased immunogenicity.

Nucleic acid vaccines have the potential to be highly amenable to use where rapid response and rapid adaptation is needed. This has clearly been demonstrated by the mRNA COVID vaccines that were rapidly advanced from discover to licensure. The DNA vaccine used in this study was not formulated with lipid nanoparticles and did not re-quire any adjuvant or electroporation. The vaccine was delivered by a technology that does not require electricity and uses relatively inexpensive disposable needle-less syringes. These devices developed by PharmaJet are FDA 510-k cleared and the Tropis device is currently utilized by Zydus Cadila Healthcare for their three dose COVID DNA vaccine that has been approved for emergency use in India. Our data confirm the immunogenicity of simple nCoV-SARS-2 DNA delivered by jet injection. For the DNA vaccine described herein, increases in dose or other steps to increase potency are warranted for a standalone vaccine; however, it is possible that the current configuration could be used to rapidly boost existing immunity using DNA vaccines encoding VOC such as Omicron.

Supporting information

S1 Data.
(DOCX)

Acknowledgments

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References

1. Coronavirus, WHO Novel. "Situation Report-51. 2020." (2019).

2. Status of Covid-19 Vaccines within Who EUL/PQ Evaluation Process. World Health Organization, 03 Mar. 2022, https://extranet.who.int/pqweb/key-resources/documents/status-covid-19-vaccines-within-who-eulpq-evaluation-process.

3. "Who Lists 9th Covid-19 Vaccine for Emergency Use with Aim to Increase Access to Vaccination in Lower-Income Countries." World Health Organization, World Health Organization, 17 Dec. 2021, https://www.who.int/news/item/17-12-2021-who-lists-9th-covid-19-vaccine-for-emergency-use-with-aim-to-increase-access-to-vaccination-in-lower-income-countries.

4. "Covid-19 Vaccine Tracker and Landscape." World Health Organization, World Health Organization, 01 Mar. 2022, https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines.

5. Malik Jonaid Ahmad, et al. "Targets and strategies for vaccine development against SARS-CoV-2." Biomedicine & Pharmacotherapy (2021): 111254. https://doi.org/10.1016/j.biopha.2021.111254 PMID: 33550049

6. Brocato Rebecca L., et al. "Protective efficacy of a SARS-CoV-2 DNA vaccine in wild-type and immunosuppressed Syrian hamsters." NPJ vaccines 6.1 (2021): 1–7. https://doi.org/10.1038/s41541-020-00279-z PMID: 33495468

7. Golden Joseph W., et al. "Human angiotensin-converting enzyme 2 transgenic mice infected with SARS-CoV-2 develop severe and fatal respiratory disease." JCI insight 5.19 (2020). https://doi.org/10.1172/jci.insight.142032 PMID: 32841215

8. Brocato Rebecca L., et al. "Disruption of adaptive immunity enhances disease in SARS-CoV-2-infected Syrian hamsters." Journal of Virology 94.22 (2020): e01683–20. https://doi.org/10.1128/JVI.01683-20 PMID: 32900822

9. Kwilas Steve et al. "A hantavirus pulmonary syndrome (HPS) DNA vaccine delivered using a spring-powered jet injector elicits a potent neutralizing antibody response in rabbits and nonhuman primates."

Current gene therapy vol. 14,3 (2014): 200–10. https://doi.org/10.2174/1566523214666140522122633 PMID: 24867065

10. Johnston Sara C., et al. "Development of a coronavirus disease 2019 nonhuman primate model using airborne exposure." PloS one 16.2 (2021): e0246366. https://doi.org/10.1371/journal.pone.0246366 PMID: 33529233

11. Shafaati Maryam, et al. "A brief review on DNA vaccines in the era of COVID-19." Future Virology 17.1 (2022): 49–66.

12. Smith Trevor RF, et al. "Immunogenicity of a DNA vaccine candidate for COVID-19." Nature communications 11. 1 (2020): 1–13. https://doi.org/10.1038/s41467-020-16505-0 PMID: 32433465

13. Dey Ayan, et al. "Immunogenic potential of DNA vaccine candidate, ZyCoV-D against SARS-CoV-2 in animal models." Vaccine 39.30 (2021): 4108–4116. https://doi.org/10.1016/j.vaccine.2021.05.098 PMID: 34120764

14. Liu Margaret A. "A comparison of plasmid DNA and mRNA as vaccine technologies." Vaccines 7.2 (2019): 37. https://doi.org/10.3390/vaccines7020037 PMID: 31022829

15. Lassaunière et al. "Preclinical evaluation of a candidate naked plasmid DNA vaccine against SARS-CoV-2." NPJ Vaccines. (2021) 6:156.

16. Patel A, et al. "Intradermal-delivered DNA vaccine induces durable immunity mediating a reduction in viral load in a rhesus macaque SARS-CoV-2 challenge model. Cell Rep Med. 2021 Oct 19; 2 (10):100420. https://doi.org/10.1016/j.xcrm.2021.100420 PMID: 34604818

17. Yu Jingyou et al. "DNA vaccine protection against SARS-CoV-2 in rhesus macaques." Science (New York, N.Y.) vol. 369,6505 (2020): 806–811. https://doi.org/10.1126/science.abc6284 PMID: 32434945

18. Momin Taufik, et al. "Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India." EClinicalMedicine 38 (2021): 101020. https://doi.org/10.1016/j.eclinm.2021.101020 PMID: 34308319