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Crimean-Congo hemorrhagic fever virus replicon particle vaccine is safe and elicits functional, non-neutralizing anti-nucleoprotein antibodies and T cell activation in rhesus macaques

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Abstract

Advancement of vaccine candidates that demonstrate protective efficacy in screening studies necessitates detailed safety and immunogenicity investigations in pre-clinical models. A non-spreading Crimean-Congo hemorrhagic fever virus (CCHFV) viral replicon particle (VRP) vaccine was developed for single-dose administration to protect against disease. To date, several studies have supported safety, immunogenicity, and efficacy of the CCHF VRP in multiple highly sensitive murine models of lethal disease, but the VRP had yet to be evaluated in large animals. Here, we performed studies in non-human primates to further evaluate clinical utility of the VRP vaccine. Twelve adult male and female rhesus macaques were vaccinated intramuscularly and clinical monitoring was performed daily for 28 days. At 3, 7, 14, 21, and 28 days post vaccination,

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Potential conflicts of interest

All authors: no reported conflicts.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.antiviral.2024.106045>.

CRediT authorship contribution statement

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animals were sedated for more detailed clinical assessment; for quantification of vaccine presence in blood and mucosal samples; and for evaluation of hematology, plasma inflammatory markers, and immunogenicity. Consistent with findings in mice, vaccination was well tolerated, with no clinical alterations nor indication of vaccine spread or shedding. In addition, vaccination induced both humoral and cell-mediated responses, with immune profile and kinetics also corroborating data from small animal models. These studies provide key data in non-human primates further supporting development of the VRP for human clinical use.

Keywords

Crimean-Congo hemorrhagic fever virus; Vaccine; Safety; Immune response; Non-human primate

1. Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV; family *Nairoviridae*, genus *Orthonairovirus*) is a tick-borne zoonotic agent that causes a spectrum of clinical signs ranging from mild to severe hemorrhagic disease, with case fatality rates of 0–40%. It has a wide endemic region, encompassing Europe, Asia, and Africa (Bente et al., 2013). Clinical disease begins with non-specific signs and symptoms, including fever, headaches, myalgia, photophobia, sore throat, nausea, vomiting and diarrhea, and/or conjunctivitis. Hemorrhagic manifestations are limited to a subset of cases and may include mild petechial rash progressing to severe and widespread ecchymoses (Bozkurt et al., 2024). Due to increasing incidence of infections and lack of FDA-approved vaccines or therapeutics, CCHFV is considered a WHO priority pathogen.

Animal models of CCHFV infection and disease currently used for vaccine safety and efficacy studies include mice (Garrison et al., 2017; Rodriguez et al., 2019; Scher et al., 2023; Zivcec et al., 2018) and non-human primates (NHPs), including cynomolgus and rhesus macaques (Hawman et al., 2021, 2023, 2024). Lethal models of Crimean-Congo hemorrhagic fever (CCHF) are limited to mice but require virus adaptation, removal of type I interferon (IFN) signaling (e.g., IFNAR^{-/-} and STAT-1^{-/-} mice) (Bente et al., 2010; Zivcec et al., 2013), or transient immunosuppression of type I IFN responses in immunocompetent mouse strains by using a monoclonal antibody (mAb 5A3) targeting the IFNAR-1 subunit of the IFN-alpha/beta receptor (Garrison et al., 2017). Early CCHFV infection studies in rhesus macaques (*Macaca mulatta*), the common patas monkey (*Erythrocebus patas*), and a baboon (*Papio* spp.) noted a lack of clinical signs (Fagbami et al., 1975; Smirnova, 1979). More recent studies also found that infection in rhesus macaques and African green monkeys (*Chlorocebus aethiops*) resulted in no overt disease. However, in 2018, a new cynomolgus macaque (*Macaca fascicularis*) disease model was reported (Haddock et al., 2018); infection led to clinical alterations such as thrombocytopenia, hypoproteinemia, edema and epistaxis, and development of severe end-point disease in 1 of 4 (25%) animals inoculated subcutaneously (SC) and intravenously (IV) and in 3 of 4 (75%) animals inoculated only IV. Subsequent studies using similar experimental approaches have failed to replicate these observations and instead indicate that cynomolgus macaques, like other NHP species reported prior and since, remain models of asymptomatic infection or mild/moderate

disease (Cross et al., 2020; Smith et al., 2019). Despite the absence of overt CCHF signs in most NHPs, viremia and consistent clinical pathology alterations (e.g., leukopenia and thrombocytopenia) have been noted in infected NHPs, including cynomolgus (Cross et al., 2020) and rhesus (Hawman et al., 2024) macaques. These data support close monitoring of clinical analytes as a tool to evaluate safety and efficacy of CCHFV-targeted vaccines in large animals, as shown in recent vaccine trials (Hawman et al., 2021, 2023, 2024).

We previously reported development and evaluation of a CCHFV viral replicon particle (VRP) vaccine. Single-dose vaccination conferred 100% protective efficacy against homologous and heterologous CCHFV challenge in IFNAR^{-/-} mice when given one month before infection (Scholte et al., 2019; Spengler et al., 2019), and protected against heterologous challenge when administered as soon as 3 days before infection (Spengler et al., 2021). Overall, the VRP serves as a highly efficacious vaccine candidate with utility for high-risk populations and outbreak response. To date, however, CCHF VRP safety and immunogenicity studies have been limited to mice (Scholte et al., 2019, 2023; Sorvillo et al., 2024; Spengler et al., 2019, 2021). NHP studies are a critical step in biomedical research because of NHP's similarity to humans in physiology, neuroanatomy, reproduction, development, cognition, and social complexity (Phillips et al., 2014). Here, we continue critical evaluation and advancement of the CCHF VRP vaccine by investigating safety and immunogenicity in rhesus macaques.

2. Materials and methods

Biosafety and ethics statement.

Experiments involving CCHFV were conducted in the BSL-4 laboratory at the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). Experiments involving cDNA-encoding viral sequences were performed in accordance with approved Institutional Biosafety Committee protocols. Animal studies were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* and approved by the CDC Institutional Animal Care and Use Committee (IACUC; #3342). The CDC is fully accredited by AAALAC International.

Cell lines.

Vero-E6 cells stably expressing the codon-optimized Oman-98 GPC were described previously (Scholte et al., 2023). BSR-T7/5 cells were a kind gift of K.K. Conzelmann (Ludwig-Maximilians-Universität, Munich, Germany). Vero-E6, BSR-T7/5, and Huh7 cells were cultured in DMEM supplemented with fetal calf serum, non-essential amino acids, sodium pyruvate, L-glutamine, penicillin/streptomycin, and puromycin (Vero-E6 Oman-98 GPC cells only). THP-1 cells (ATCC TIB-202) were cultured in RPMI supplemented with fetal calf serum and antibiotics, as above.

Vaccine stock production and characterization.

CCHF VRP stocks were generated as previously described (Scholte et al., 2019). Briefly, CCHF VRPs were generated by transfecting HuH-7 cells with plasmids encoding CCHFV strain IbAr10200 S and L genomic segments (pT7-S and pT7-L; GenBank [KJ648914](#)

and [KJ648913](#)) and strain Oman-98 glycoprotein (pCAGGS-GPC-Oman; GenBank [ALT31693.1](#)) along with plasmids encoding the codon-optimized polymerase (pCAGGS-L), nucleoprotein (pCAGGS-NP), and T7 polymerase (pCAGGS-T7). Supernatants were propagated and quantified via immunofluorescent TCID₅₀ assay using Vero-E6 cells stably expressing the codon-optimized Oman-98 GPC; immunostaining was performed using a rabbit polyclonal anti-NP CCHFV antibody (1:2500; IBT, 04-0011) followed by a goat anti-rabbit secondary antibody (1:2500; Alexa Fluor 488, Invitrogen, A-11008). Wells were scored visually for the presence or absence of fluorescent cells, and TCID₅₀ values were calculated using the Reed-Muench method (Reed and Muench, 1938). All vaccine stocks were verified to be mycoplasma free (MycoAlert PLUS detection kit, Lonza LT07), and genomic sequences were confirmed via next-generation sequencing prior to in vivo studies. Backtiter quantification of vaccine inoculum used on day of vaccination (day 0) was performed as described above.

Rhesus macaques.

For these studies, a healthy heterogeneous cohort of 12 adult rhesus macaques (6 males [5.8–11.1 kg] and 6 females [4.3–6.8 kg]; all 4–5 years of age) was selected based on previous clinical history and records and on current health parameters/status (i.e., normal values on recent health assessments, negative for simian immune deficiency virus, and no chronic conditions or health concerns). For $\alpha = 0.05$ and a difference of 1.25 standard deviations, 12 animals provide a power of 80%. Animals were social/pair housed in a climate-controlled laboratory with a 12 h day/night cycle in standard NHP cages, and given NHP chow (High Protein Monkey Diet, Labdiet 5045) and water ad libitum. Per standard institution practices, animals were also provided daily produce enrichment items including fruits and/or vegetables and received destructible enrichment/treats twice per week. Animals were kindly provided for use in this non-terminal study by the Laboratory Branch of the CDC Division of HIV Prevention and transferred back to originating protocols upon study completion. Animals had previously been used in hepatitis B vaccine studies, and to assess doxycycline vaginal inserts in a vaginal chlamydia challenge. Vaginal swabs from all 6 chlamydia-challenged females tested negative for chlamydia over 3 months prior to study initiation. Female and male rhesus monkeys had not been actively on study for approximately 4 and 10 months, respectively, before CCHF VRP vaccination.

Sedation, vaccination, and recovery monitoring.

For vaccination, blood collection, and mucosal swabbing, animals were anesthetized with ketamine (2–10 mg/kg) intramuscularly (IM) in the cranial quadriceps using a 22-gauge needle. After anesthesia, animals were monitored every 15 min until they were ambulatory and alert, indicating full recovery. On vaccination day, the fur on the left or right lateral thigh was clipped, and vaccine was administered IM with a 23-gauge, 1-inch needle in the clipped area (target dose: 1×10^6 TCID₅₀; backtiter dose: 7×10^5 TCID₅₀; 1 mL total volume).

Venipuncture.

Prior to vaccine inoculation, animals were sedated, and 5 mL blood was collected from the femoral vein using a 5 mL syringe with a 23-gauge, 1-inch needle. The needle was removed, and the blood was divided into 3 different collection tubes: ~500 μ L each into an

EDTA tube (BD Microtainer with K2E; 365974) and a lithium/heparin (LiH) tube (Sarstedt Micro tube 1.3 mL LH; 41.1393.105), and ~4 mL into a sodium citrate CPT Vacutainer (BD Biosciences; 362760). Blood draw volumes were in compliance with IACUC policy (single sampling $\leq 10\%$ of total body weight [TBV]; $\leq 7.5\%$ TBV taken weekly; repeated serial sampling $\leq 7.5\%$ TBV within a 7-day period).

Non-invasive clinical monitoring, clinical chemistry, and complete blood chemistry.

Daily mentation/activity levels were recorded as bright alert reactive (BAR) or quiet alert responsive (QAR), along with any other descriptors of activity noticed during assessment. Hydration status was recorded as adequate (no sunken eye appearance, skin laxity, or droopiness) or inadequate (mild, moderate, or severe based on sunken eye appearance or skin looseness/tenting as observed cage-side). Heart rate and electrocardiogram (ECG) monitoring were recorded using an Avante DRE ASM 5000 monitor. Respiration was assessed via auscultating the lungs, and SpO₂ was recorded with a NONIN 8600V pulse oximeter. Body temperatures were recorded with a rectal thermometer probe (Welch Allyn SureTemp Plus 690 handheld electronic thermometer). Clinical chemistry was analyzed on whole-blood samples collected in LiH using Piccolo Xpress chemistry analyzers with the Comprehensive Metabolic Panel (Abaxis). Complete blood counts (CBC) were performed on whole-blood samples collected in EDTA using the Vetscan HM5 hematology analyzer (Abaxis).

RT-qPCR.

RNA was extracted from whole blood collected in EDTA (50 μ L), and from oral, nasal, and rectal mucosal swabs collected using sterile polyester-tipped applicators (Puritan; 25–800 1PD 50) placed in 1 mL of MagMAX lysis buffer. MagMAX Pathogen RNA/DNA kits (Thermo Fisher Scientific 4462359) were used in conjunction with the 96-well ABI MagMAX extraction platform; RNA was eluted into 75 μ L of elution buffer. Viral RNA was quantified using a primer/probe set targeting the NP open reading frame of the S genomic segment of CCHFV strain IbAr10200 (forward: 5'-CAG GAC ATG GAC ATA GTG GC-3'; reverse: 5'-ATT GCC CTT GAC GTT GTA GG-3'; probe: 5'-CCC TTG TTG GCA AGC AAT CCC-3' [all IDT]) using the SuperScript III Platinum One-Step RT-qPCR kit (Thermo Fisher 11732088). Tissue RNA levels were normalized using the validated reference gene *Ppia* (Davies et al., 2023). Viral RNA copy numbers were quantified via standard curves generated from an RNA standard of known concentration (IDT).

Cytokine/chemokine analyses.

EDTA plasma samples were analyzed from each animal 0, 3, 7, 14, and 21 days post vaccination. Plasma was heat-inactivated at 56 °C for 30 min and analyzed (25 μ L) using the ProcartaPlex NHP Cytokine and Chemokine 30-plex panel (Thermo Fisher EPX300–40044-901) according to the manufacturer's instructions. Data were generated using the Luminex xMAP INTELLIFLEX system and analyzed using the ProcartaPlex Analysis App software (Thermo Fisher). All analytes were calculated based on standard curves and reported as mean fold change in mean fluorescence intensity compared to baseline (day 0) values (Fig. 2C), or as absolute values (pg/mL, Supplemental Fig. 1).

Serology.

CCHFV anti-glycoprotein-specific (Gn, Gc, and GP38), and anti-NP-specific IgG antibodies were detected as described previously (Scholte et al., 2023). Briefly, Immulon 2HB 96-well microtiter plates were coated with 100 μ L of 500 ng/mL antigens prepared in PBS and incubated overnight at 4 °C. Wells were washed 4 $\times$ with 300 μ L PBS-T (0.1% Tween-20 in PBS) and blocked (5% w/v non-fat dry milk in PBS-T) for 1 h at room temperature. Following blocking, heat-inactivated plasma samples prepared in blocking buffer with 2-fold serial dilutions (range 1:200 to 1:102,400) were added to the wells in duplicate. After 1 h incubation, wells were washed, and anti-rhesus IgG linked to horseradish peroxidase (HRP; 1:3000; Southern Biotech 4700–05) was added to the wells. Following 1 h incubation, wells were washed, TMB Ultra ELISA substrate (Thermo Fisher 34028) was added, and samples were incubated for 20 min. The reaction was stopped by adding ELISA stop solution (Thermo Fisher SS040), and optical density was read at 450 nm on a Synergy Neo2 instrument (BioTek) microplate reader. A cut-off value was determined for each plate based on the average absorbance value of 12 negative control wells per plate plus 5 standard deviations. The highest dilutions with a signal above the determined cut-off value were assigned as the endpoint titers. For the detection of antigen-specific IgG subclasses, mouse anti-rhesus IgG1-specific (Absolute Antibody Ab01623–4.0), IgG2-specific (Creative Biolabs NHP-AB055), IgG1/3-specific (Creative Biolabs NHP-AB052), and IgG4-specific (Absolute Antibody Ab01624–1.1) antibodies were used in 1:3000 dilution along with anti-mouse IgG-HRP (Invitrogen 61–6520). For the detection of antibody avidity, ELISAs were performed as described above with an additional step. Following 1 h incubation of plasma samples and washing, replicates of samples were incubated with PBS (untreated) or with 6 M urea (treated) for 10 min. The assay was completed as described above, and the area under the curves (AUCs) was calculated for untreated and treated wells. The avidity index was determined by dividing the AUC of the urea-treated wells by that of the PBS-treated wells and then multiplying by 100 (Almanzar et al., 2013).

Fc-mediated effector function.

Antibody-dependent complement deposition (ADCD) and antibody-dependent cellular phagocytosis (ADCP) assays were adapted from previously reported methods (Butler et al., 2019; Fischinger et al., 2019). Recombinant CCHFV NP generated based on strain Hoti was biotinylated (EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit, Thermo Fisher 21435) and coupled to fluorescent red or green neutravidin microspheres (Thermo Fisher F8775/F8776). Antigen-coated beads were incubated 2 h at 37 °C with heat-inactivated (30 min at 56 °C) NHP plasma in sodium citrate. For ADCD assays, guinea pig complement (Cedarlane CL4051) diluted in gelatin veronal buffer (CompTech B102) was added and incubated 15 min at 37 °C. Beads were washed and incubated for 15 min at room temperature with FITC-conjugated anti-guinea pig complement C3 (MP Biomedicals 0855385). For ADCP assays, beads were incubated overnight at 37 °C with 2×10^4 THP-1 cells per well. Beads and cells were analyzed on a Guava cytometer. Phagocytic score was calculated by multiplying the percentage of bead-positive cells by the overall median fluorescence intensity.

Peripheral blood mononuclear cell isolation and ELISpot.

Blood was collected in sodium citrate CPT Vacutainers (BD Biosciences 362760), and peripheral blood mononuclear cells (PBMCs) were isolated per the manufacturer's recommendations. Briefly, tubes were centrifuged for 15–20 min at 1500–1800×*g*. The top plasma layer was removed and stored for other downstream applications/analyses. The remaining mononuclear cells were washed twice with PBS before dilution in RPMI supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin (2 million cells/mL). PBMCs (200,000 cells/well) were stimulated with peptides spanning the CCHFV strain IbAr10200 NP protein (Abclonal; 15-mers with 9 amino acids overlap; 1 µg/mL; 90% purity). PBMCs were mixed with stimuli and seeded in IFN-gamma ELISpot plates (MabTech 3421M-4HPW). DMSO was used as a negative control and phorbol myristate acetate (PMA)/ionomycin (BioLegend, 423302) was used as a positive control. PBMCs to be stimulated with PMA/ionomycin were diluted 1:20 (10,000 cells/well) to prevent confluence and ensure countable spots. PMA/ionomycin was used at the concentration recommended by the manufacturer (1:500; 0.05 µg/mL PMA and 1 µg/mL ionomycin final concentration). IFN-gamma spots were counted with a CTL ELISpot reader and normalized to PMA-induced spots. Antigen-specific spots were determined by subtracting spots in the DMSO-treated wells.

Neutralization.

Sodium citrate plasma samples were heat-inactivated (30 min at 56 °C) and 2-fold serially diluted in DMEM (starting dilution 1:10). Diluted plasma samples were mixed with an equal volume of DMEM containing 200 TCID₅₀ of recombinant CCHFV reporter virus expressing ZsGreen1 fluorescent protein (Oman-ZsG; total volume: 100 µL). Mixtures were incubated 1 h at 37 °C and subsequently overlaid onto BSR-T7 cells seeded in 96-well plates. Cells were incubated 5–7 days, and ZsG-positive wells were scored to determine neutralizing activity. Mouse monoclonal antibodies (mAbs; 11E7 and 30F7) were used as a positive control. 11E7 and 30F7 mouse mAbs were obtained from the Joel M. Dalrymple-Clarence J. Peters USAMRIID Antibody Collection through BEI Resources, NIAID, NIH: Pre-Gc Glycoprotein, Clone 30F7 (NR-40288) and Clone 11E7 (NR-40277).

Data visualization.

All graphs were generated using GraphPad Prism version 10.2.3. Fig. 1A was created with BioRender.com.

3. Results

3.1. CCHF VRP vaccine is safe in non-human primates

Twelve rhesus macaques (adult; 6 males, 6 females) were vaccinated IM with CCHF VRP. Daily cage-side evaluations were performed until study completion (28 days post vaccination) to assess mentation, activity level, hydration status, and the injection site. Fur at the injection site was clipped prior to vaccination to facilitate evaluation for papules, redness, bruising, and other signs of injection site reactions. The injection site was examined

cage side 2–3 h post vaccination, when acute injection site reactions were most likely to occur, and then daily afterwards.

A total of 7 sedation events were performed over the course of the study, on days –4, 0, 3, 7, 14, 21, and 28 post vaccination (Fig. 1A). During sedation events, blood and non-invasive clinical data (weight, body temperature, respiration, heart rate, SpO₂, and ECG) were collected. Two baseline measurements were obtained for CBC and chemistry, along with non-invasive clinical data 4 days prior to vaccination (day –4), and immediately prior to vaccination on day 0. All clinical data were evaluated independently by two licensed and ACLAM-boarded clinical veterinarians experienced with NHPs. Post-vaccination clinical findings were largely unremarkable (Fig. 1B). Throughout the 28-day study period, mentation/activity level were recorded as within normal limits, hydration status was adequate, and no injection site-associated erythema or swelling were observed. Four of the twelve animals lost weight (max weight loss <10% from baseline). While weight loss due to vaccination alone cannot be ruled out, animals under daily study observations and sedated ≥ once weekly often exhibit weight loss; all changes were within expected limits for the frequency of handling and sedation. No abnormalities were observed cage side, except for a single observation of vomit in the cage of one animal on day 10. Investigation revealed that the animal received an uncommon enrichment food item less than 24 h prior to the vomiting episode. No other signs of GI upset or toxicity were noted. Overall, no notable deviations from baseline were observed by non-invasive sampling post vaccination, and no clinical alterations noted were considered vaccine related.

To assess safety of the vaccine, VRP dissemination (blood) and mucosal shedding (oral, nasal, and rectal swabs) were evaluated on days 0, 3, 7, 14, 21, and 28 post vaccination. All samples were CCHFV-negative by RT-qPCR, supporting the non-spreading nature of the vaccine (Fig. 1C). To assess changes in blood parameters post vaccination, complete blood counts and clinical chemistry were performed. No notable changes were seen from baseline following vaccination (Fig. 2A). At both baseline timepoints, elevated alkaline phosphatase (ALP) and decreased glucose and K⁺ were observed in a subset of animals (Fig. 2B). These values are believed to be within normal limits for the colony or associated with fasting prior to anesthesia. Increased ALP is also associated with amyloidosis deposits in the liver, which are common age-related changes resulting in liver enzyme elevation (Rice et al., 2013) and have been reported in NHPs. Additionally, inflammation was evaluated by cytokine/chemokine analysis using Luminex up to 21 days post vaccination (Fig. 2C). No clinically relevant differences from baseline (day 0) were observed in plasma cytokine/chemokine levels post vaccination.

3.2. Single-dose CCHF VRP vaccination in NHPs elicits strong anti-nucleoprotein humoral responses and T-cell activation

Previous work on the VRP characterized responses to vaccination in mice following vaccination alone (Scholte et al., 2023) and in vaccinated mice after CCHFV challenge (Sorvillo et al., 2024). Our study is the first to characterize the immune responses to VRP vaccination in NHPs. IgG responses targeting a spectrum of viral antigens were evaluated, including the glycoproteins (Gn, Gc, GP38) and nucleocapsid protein (NP). Overall, the

primary antibody responses detected were against NP: anti-NP antibodies were detected in half of the animals on day 7, in all animals on day 14, and were comparable in magnitude in all animals on days 21 and 28 (Fig. 3A). A subset of animals (9 of 12) developed anti-Gc antibodies, but no antibodies were detected against Gn or GP38.

To better characterize the IgG response, we evaluated the development of IgG1, IgG2, IgG1/3, and IgG4 subclasses of anti-NP antibodies (Fig. 3B). No IgG2 nor IgG4 were detected, but both IgG1 and IgG3 subclasses were present in 11 of 12 (91.6%), and 12 of 12 animals (100%), respectively. Additionally, anti-NP antibody avidity and function were evaluated (Fig. 3C). The overall quality of antibody binding (avidity) began to increase on day 14 and continued through day 28. NP-specific antibodies were able to bind complement and induce ADCD activity starting on day 14, with similar levels on days 21 and 28. NP-specific antibodies were also able to mediate phagocytosis, with activity detected starting on day 14. Consistent with previous VRP vaccine studies indicating low or absent neutralizing responses (Kainulainen et al., 2019; Sorvillo et al., 2024; Welch et al., 2023), very low-level neutralizing activity was observed in a small subset of animals on days 7, 14, and 21 (1:10 dilution).

Our previous CCHF VRP studies (Scholte et al., 2023; Sorvillo et al., 2024), as well as studies of VRP vaccines against Lassa (Kainulainen et al., 2018) and Nipah viruses (Welch et al., 2023), indicate a predilection for and importance of NP-targeted antibodies. Herein, we found a similar predominance of NP-mediated antibody responses in rhesus macaques. To further characterize NP responses, we assessed cell-mediated responses by ELISpot in PBMCs collected on days 0, 3, 7, 14, 21, and 28 post vaccination using NP peptides (Fig. 3D). As previously observed in mice (Scholte et al., 2023), NP-specific T cell activation (IFN-gamma expression) was detected, peaking on day 7 and waning afterwards.

4. Discussion

Previous studies in highly sensitive mouse models of CCHF disease have demonstrated safety and efficacy of the VRP vaccine (Scholte et al., 2019; Spengler et al., 2019, 2021). Rhesus macaques have high genomic homology to humans, including similar immune systems. As such, they are an advantageous model for evaluating and predicting vaccine toxicity in humans (Estes et al., 2018; Yang et al., 2023). Here, we performed comprehensive clinical assessments in rhesus macaques vaccinated with CCHF VRP to further confirm safety of this vaccine. Because this study was non-terminal, no histopathology was performed; in lieu of histopathological studies, clinical analytes were assessed as indicators of organ damage and function, and comprehensive cardiovascular exams were completed. In addition, we characterized T cell activation and antigen-specific antibody responses along with associated avidity and function up to 28 days following vaccination. Overall, we provide the first report of CCHF VRP vaccination in NHPs, presenting key safety and immunogenicity data to aid in advanced development and regulatory approval of this highly promising vaccine candidate.

Generally, CCHFV does not cause overt clinical signs in immunocompetent hosts (Garrison et al., 2019; Spengler et al., 2016). Sensitive models of severe disease are largely restricted

to approaches that immunocompromise the hosts, particularly by targeting the type-I IFN response (i.e., using hosts with immature immune systems, gene knock-out models, or models with transient immunosuppression) (Bente et al., 2010; Garrison et al., 2017; Zivcec et al., 2013). Earlier studies in NHPs supported their susceptibility to infection in the absence of overt clinical signs (Faghami et al., 1975; Hoogstraal, 1979; Smirnova, 1979). However, more recent studies have demonstrated that severe disease may occur in a subset of animals depending on NHP species, virus strain, and experimental delivery (Haddock et al., 2018). NHPs are now being used in therapeutic and vaccine efficacy studies with a focus on parameters (e.g., viremia, platelet levels, liver analytes, viral replication in tissues in terminal studies) that can consistently be evaluated to indicate therapeutic benefit (Hawman et al., 2020, 2024). Vaccine delivery in NHPs is an important step for advanced product development for clinical use. In these studies, all clinical assessments support data seen in small animal models to date, indicating a high safety profile.

Detailed CCHF VRP immunological studies were previously limited to mice. It was unclear if the characteristics and kinetics of the response would translate to other species, particularly NHPs, as an indication of putative responses in humans. In mice, the IgM and IgG antibody responses largely target NP, with minimal antibodies (especially IgG) detected against glycoproteins (Scholte et al., 2023). In rhesus macaques, antibody profiles, avidity, and function, together with the absence of notable neutralization, also parallel VRP vaccination data in mice (Scholte et al., 2023; Sorvillo et al., 2024). Importantly, our studies provide further support in NHPs for the importance of NP expression in eliciting robust antibody responses, and for functional roles of these antibodies. In recent DNA vaccine studies in cynomolgus macaques, an NP-based but not a GPC-based vaccine resulted in CCHFV-specific IgG responses (Hawman et al., 2023); when both antigens were included in vaccination, CCHFV-specific IgG responses were nearly identical to responses obtained when NP-based vaccine was used alone. We also found that ADCD and ADGP activity typically followed NP IgG kinetics, and that IgG subclass characterization aligns with functionality assays. Studies show that the least to most activating IgG subclasses in rhesus are IgG4 < IgG3 < IgG2 < IgG1, with IgG 1 being the most activating subclass (Boesch et al., 2016; Crowley and Ackerman, 2019). In this study, we detected IgG1 and IgG1/IgG3.

Overall, our data provide several key advances for the use of the CCHF VRP vaccine. Here we confirm its safety and tolerability in NHPs and demonstrate a robust immune response consistent with responses characterized in mice. These data support continued advancement of the vaccine and indicate that preliminary studies in mice may serve as reliable indicators of both parameters in other diverse species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Data availability

Data will be made available on request.

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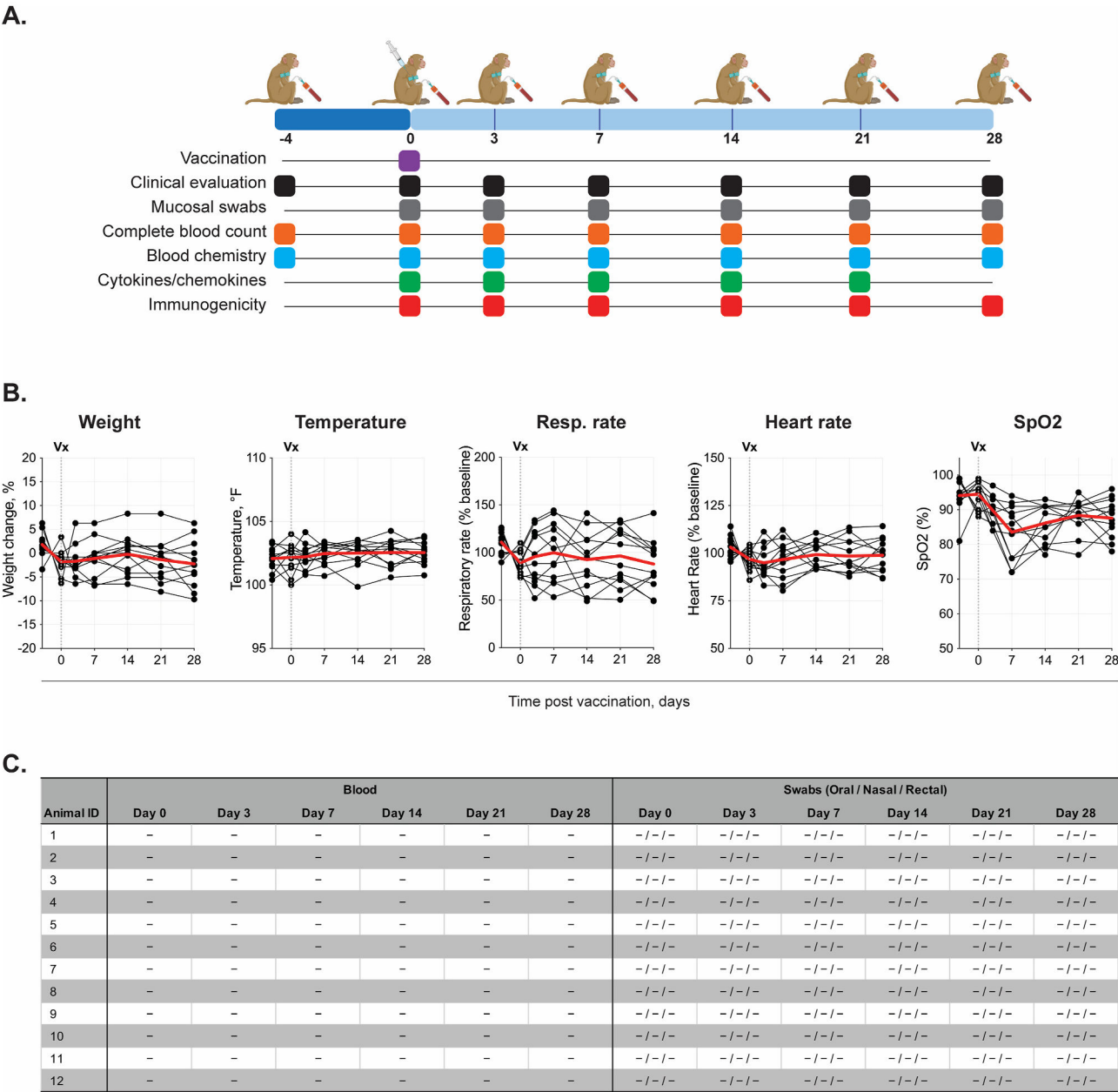


Fig. 1. (A) CCHF VRP vaccine safety and immunogenicity study design. Twelve adult rhesus macaques received a single dose of VRP intramuscularly (target dose: 1×10^6 TCID₅₀) and were followed for 28 days. Two baseline samplings were performed on day -4 and day 0 (prior to vaccination). Cage-side health observations, including injection site examination and assessment of hydration status, were performed daily. Longitudinal samplings were performed 3, 7, 14, 21, and 28 days post vaccination. (B) Changes in weight, body temperature, respiratory rate, heart rate, and SpO₂ from baseline. Baseline values were calculated as average of day -4 and day 0 values. Circles indicate individual data ($n = 12$), and thick line indicates the group mean. (C) RT-qPCR analyses confirming lack of detection

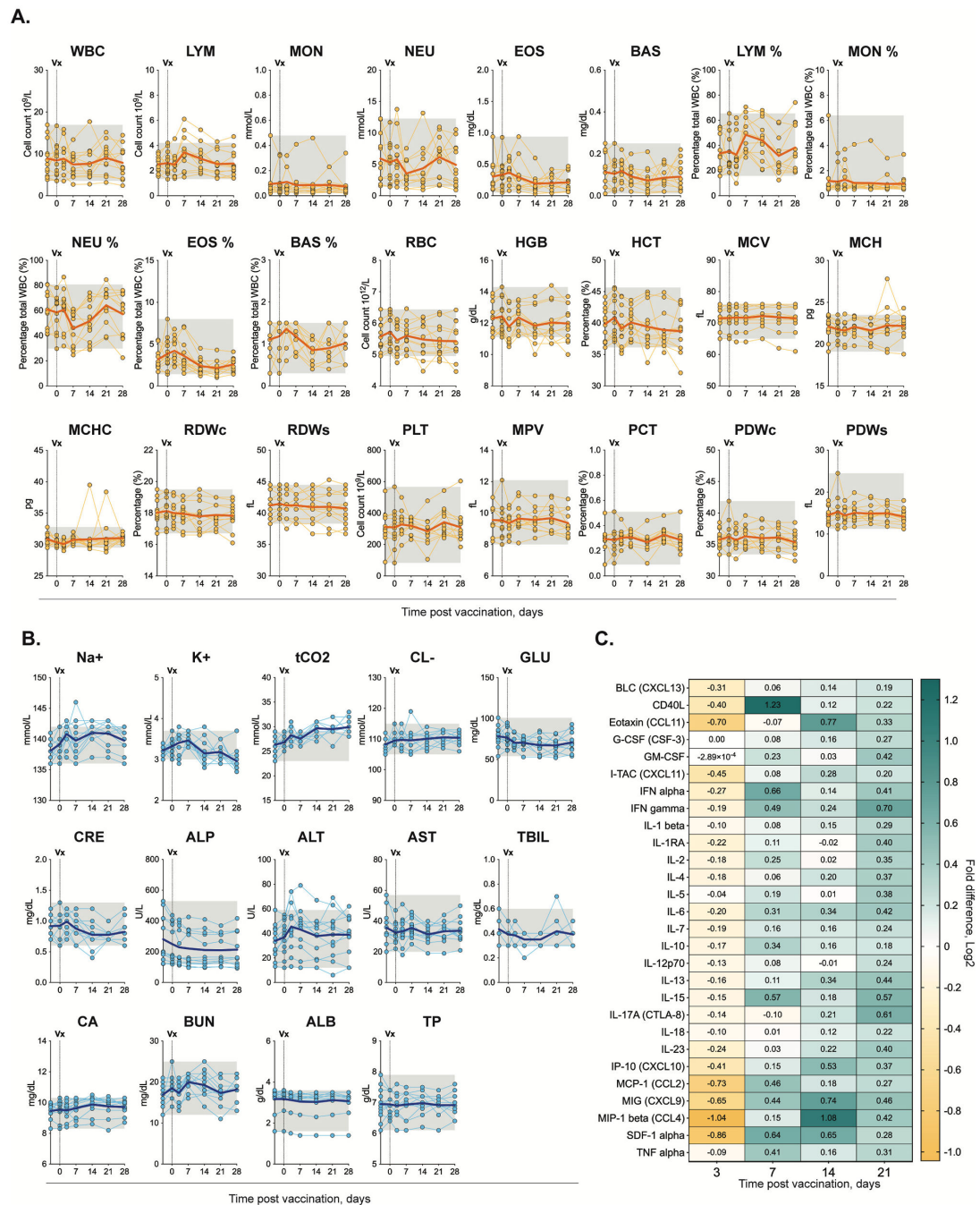
of CCHF VRP RNA in blood or oral, nasal, and rectal swab samples post vaccination to support the vaccine safety profile (no circulating vaccine or vaccine shedding).

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**Fig. 2.**

(A) Complete blood counts and (B) clinical chemistry analyses in rhesus macaques on days -4 and 0 (prior to vaccination), and on day 3, 7, 14, 21, and 28 post VRP vaccination. Baseline values were calculated as averages of day -4 and day 0 values. Circles indicate individual data ($n = 12$), and thick line indicates the group mean. Grey shading indicates range of values (minimum to maximum) obtained in baseline samplings performed on day -4 and day 0 (prior to vaccination). (C) Plasma cytokine and chemokine analyses from CCHF VRP-vaccinated rhesus macaques. EDTA plasma samples were analyzed from each

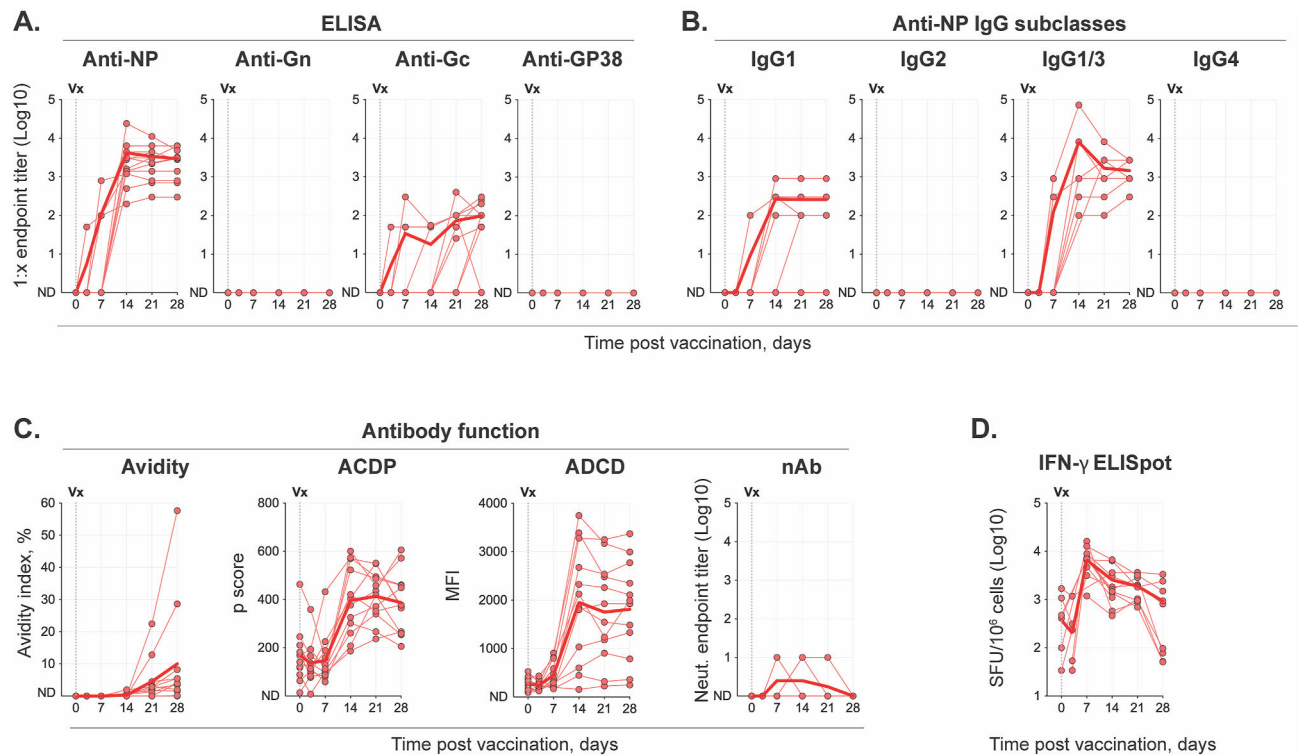
animal at baseline (day 0) and on days 3, 7, 14, and 21 post vaccination. Data for each analyte are reported as median fold change compared to baseline (day 0) values.

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**Fig. 3.**

CCHF VRP vaccine immunogenicity in rhesus macaques. (A) IgG ELISA to detect anti-NP, Gn, Gc, and GP38 antibodies. (B) IgG subclass analyses. (C) Assessment of antibody avidity index and function. The avidity index, expressed as a percentage, was determined by dividing the area under the curve (AUC) of the urea-treated wells by that of the PBS-treated wells and then multiplying by 100. (D) IFN- γ assessment by ELISpot. ACDP, antibody-dependent cellular phagocytosis; ADCD, antibody-dependent complement deposition; nAb, neutralizing antibody; ND, not detected; P score, phagocytic score; SFU, spot forming unit.