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Supplementary Materials for

VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain

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Materials and Methods

Animal ethics and biosafety statement

All macaque work was performed in strict accordance with the recommendations described in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health, the Office of Animal Welfare and the United States Department of Agriculture. Animal procedures were carried out under anesthesia by trained personnel under the supervision of veterinary staff and all efforts were made to promote the welfare and to minimize animal suffering in accordance with the "Weatherall report for the use of non-human primates" recommendations. Animals were housed in adjoining individual primate cages allowing social interactions, under controlled conditions of humidity, temperature and light (12-hour light/12-hour dark cycles). Food and water were available ad libitum. Animals were monitored at least twice daily (pre- and post-infection) and fed commercial monkey chow, treats and fruit twice daily by trained personnel. Environmental enrichment consisted of commercial toys and music. Humane endpoint criteria, specified and approved by the Institutional Animal Care and Use Committee (IACUC), were applied to determine when animals should be humanely euthanized. All infectious animal work was performed in the maximum containment laboratory at the Rocky Mountain Laboratories (RML), Division of Intramural Research (DIR), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Montana, USA applying standard operating protocols approved by the Institutional Biosafety Committee (IBC).

Vaccine and challenge virus

GMP-grade VSV-EBOV, expressing the glycoprotein (GP) of the EBOV-Kikwit strain, was manufactured by KBP (Germany) under the ownership of the Public Health Agency of Canada and used to immunize macaques at a concentration of 5x 10^7 plaque-forming units (PFU)/ml. Non-GMP-grade VSV-MARV, expressing the glycoprotein (GP) of the MARV-Angola strain was produced by concentrating and purifying tissue culture supernatant and to immunize macaques at a concentration of 5x 10^7 PFU/ml. EBOV-Makona (Guinea C07; passage 1) *(1, 11),* EBOV-Kikwit (passage 1) and EBOV-Mayinga (passage 5) *(11)* (the initial inoculation of cell cultures with clinical material is regarded as passage 0) were propagated on Vero E6 cells (mycoplasma negative), titrated on these cells and stored in liquid nitrogen. Deep sequencing confirmed that the predominant phenotype for all three distinct EBOV strains was 7U, representing wild-type EBOVs and not the tissue culture-adapted 8U phenotype *(28)*.

Study design

A total of 15 cynomolgus macaques (*Macaca fascularis*), 11 male and 4 female animals, 6-10 years of age and 4-7 kg in weight, were used in this study. The study was not blinded and macaques were randomly divided into 5 study groups (n=2 or n=3) and one control group (n=3). The 12 study animals were immunized intramuscularly at one site in the left caudal thigh with 5x 10^7 PFU GMP-grade VSV-EBOV (0.5 ml inoculum). The control animals received the same dose of VSV-MARV via the same route. All animals were challenged intramuscularly on day 0 with a lethal dose of 1,000 PFU EBOV-Makona (0.5 ml inoculum; confirmed by back-titration) at one site in the right caudal thigh. Physical examinations and blood draws were performed on days -28, -21, -14, -7, -3, 0, 3, 6, 9, 14, 21, 28, 35 and 42 as well as at the time of euthanasia. The animals were

observed at least twice daily for clinical signs of disease according to an IACUC approved scoring sheet. A score (0-15) was assigned for general appearance, skin and fur, nose/mouth/eyes/head, respiration, feces and urine, food intake, and locomotor activity. These scores were recorded on a daily observation sheet and animals were euthanized by trained and experienced personnel when the total value reached the critical number of 35, or any of the following signs were observed: impaired ambulation preventing access to food or water; excessive weight loss; lack of mental and physical alertness; difficult labored breathing or prolonged inability to remain upright.

Virus loads

For determination of virus loads in macaque blood and tissue samples, Vero E6 cells (mycoplasma negative) were seeded in 48-well plates the day before titration. Blood samples were thawed and 10-fold serial dilutions were prepared. Tissues were homogenized in 1 ml plain DMEM and 10-fold serial dilutions were prepared. Media was removed from cells and triplicate wells were inoculated with each dilution. After one hour, DMEM supplemented with 2% FBS, penicillin/streptomycin and L-glutamine was added and cells were incubated at 37°C. Cells were monitored for cytopathic effect (CPE) and 50% tissue culture infectious dose ($TCID_{50}$) was calculated for each sample employing the Reed and Muench method.

Hematology and serum chemistries

The total white blood cell count, lymphocyte, neutrophil, platelet, reticulocyte and red blood cell counts, hemoglobin, and hematocrit values were determined from EDTA blood with the IDEXX ProCyte DX analyzer (IDEXX Laboratories). Serum biochemistry was

analyzed using the Piccolo Xpress Chemistry Analyzer and Piccolo General Chemistry 13 Panel discs (Abaxis).

Humoral immune responses

Enzyme-linked immunosorbent assay (ELISA) plates were coated with EBOV-GPΔTM antigen (IBT Bioservices) overnight at 4^{o}C as previously described *(6)*. After three washes with PBS/0.05% Tween, serial 4-fold dilutions of the serum samples were incubated in duplicate for 1h at 37^{o}C. Following washing three times with PBS/0.05% Tween, horseradish peroxidase (HRP)-conjugated anti-monkey IgG (KPL) was added for 1h, followed by additional washes and final addition of substrate (KPL). IgG endpoint titers were calculated using log-log transformation of the linear portion of the curve, and 0.1 optical density (OD) units as cut-off. IgG titers were standardized using a positive control sample that was included on every ELISA plate.

EBOV-GP specific IgM responses were assessed using a commercial ELISA kit (Alpha Diagnostics) for those animals with no evidence for IgG antibodies at the time of challenge (VSV-MARV vaccinated control, day -7 and day -3 animals). Serum samples were assayed in duplicates in a 1:400 dilution and EBOV-GP specific IgM concentrations were calculated according to the manufacturers instructions.

Neutralizing antibody titers were determined by focus reduction neutralization titration assays. Briefly, Vero E6 cells (mycoplasma negative) were seeded into 96-well plates to generate a confluent monolayer on the day of infection. Two-fold serum dilutions were prepared in triplicate in plain DMEM and 25 µl were incubated with 200 PFU EBOV-Makona, EBOV-Kikwit or EBOV-Mayinga in a total volume of 50 µl for 60 min at 37ºC. Media was removed from cells, the serum-virus mixture was added and samples were

incubated for further 60 min at 37ºC. The mixture was removed from the cells and 100 µl of 1.2% carboxymethyl cellulose in MEM (2% FBS) was added per well and left for 4 days at 37°C. The cells were fixed in 10% neutral buffered formalin and removed from the maximum containment laboratory according to approved standard operating procedures (SOPs). Foci were stained using an anti-EBOV-VP40 polyclonal rabbit serum kindly provided by Yoshihiro Kawaoka (University of Wisconsin-Madison) *(11)* and a secondary anti-rabbit FITC antibody (Sigma). Foci were counted and the neutralizing activity was determined as percent reduction of EBOV infection compared to control infected cells without serum.

Serum cytokine levels

Macaque sera were inactivated by γ-irradiation (5 MRad) and removed from the maximum containment laboratory according to approved SOPs. Serum samples were then diluted 1:2 in serum matrix for analysis with Milliplex Non-Human Primate Magnetic Bead Panel as per manufacturer's instructions (Millipore Corporation). Concentrations for IFNγ, IL-1β, IL-6, IL-8, IL-10, IL-15, IL-18, granulocyte colony-stimulating factor (G-CSF), MCP-1, and TGFα were determined for all samples using the Bio-Plex 200 system (BioRad Laboratories Inc.). Levels of IFNα were determined using the Cynomolgus/Rhesus IFN Alpha ELISA Kit (PBL Assay Science) following the manufacturer's instructions.

VSV detection

Total RNA was isolated from 140µl EDTA blood samples at the day of challenge using the QIAmp viral Mini RNA kit (Qiagen). All quantitative real-time RT-PCRs were performed with the QIAquick 1-step Rotorgene kit (Qiagen) and VSV-specific primers

and probes based on the nucleoprotein sequence: probe FAM-CGCCACAAGGCAG-MGB; forward primer CGGAGGATTGACGACTAATGC; reverse primer CGAGCCATTCGACCACATC.

Statistical analyses

Two-way ANOVA with Tukey multiple comparison post test was used to determine statistical significance at the level of 0.05 or lower. In addition, the Kaplan-Meier method was employed to analyze statistical significance for survival at the level of 0.05 or lower. All analyses were carried out using Prism Software.

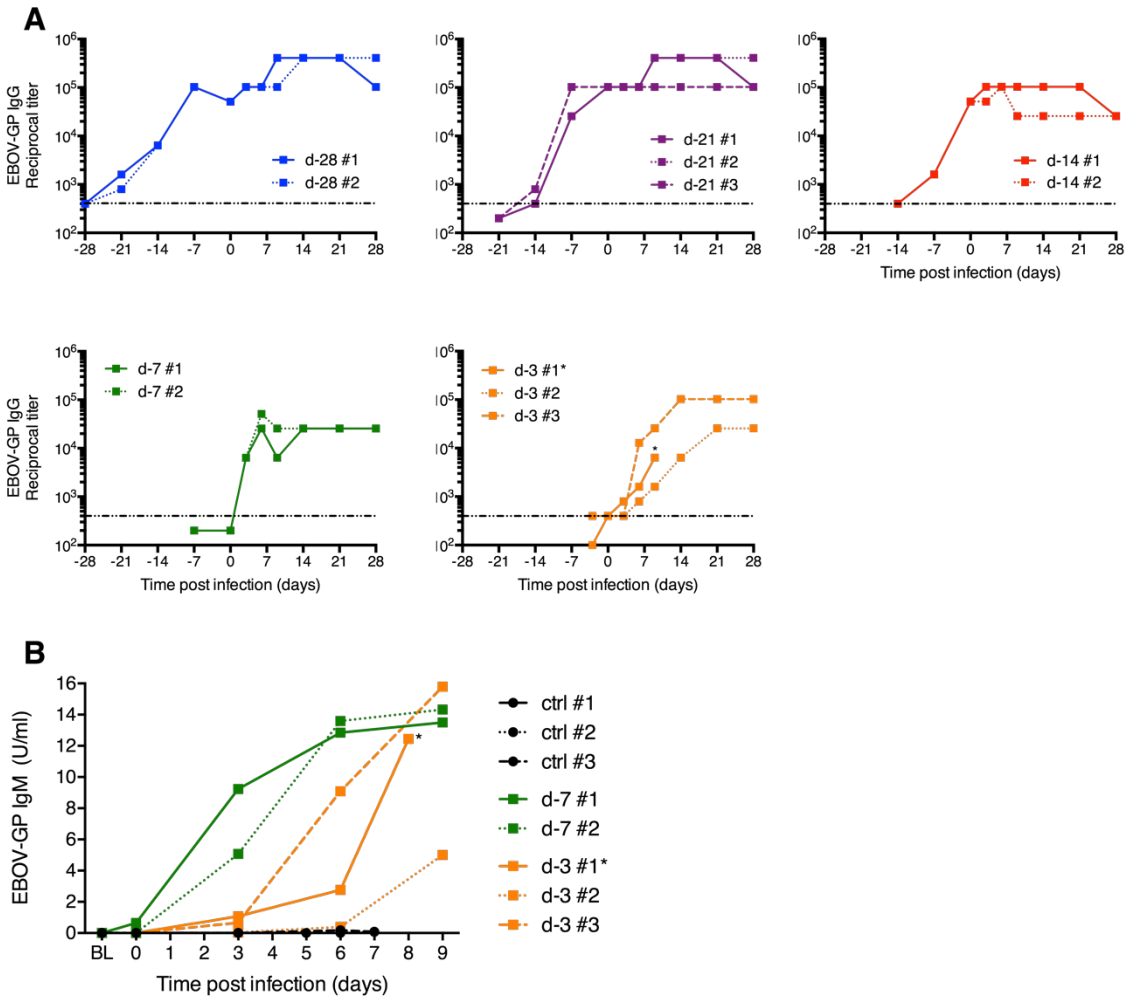

Fig. S1: EBOV-GP specific IgG and IgM titers determined by ELISA. (A) For each individual animal EBOV-GP specific IgG titers were determined in serum at examination days. The dotted line marks the ELISA background level based on titers obtained from negative control animals. (B) Serum of the animals in the VSV-MARV vaccinated control and the day -7 and day -3 VSV-EBOV vaccinated groups was analyzed for EBOV-GP specific IgM at the indicated time points. BL = base line. *Animal d-3 #1 euthanized on day 8.

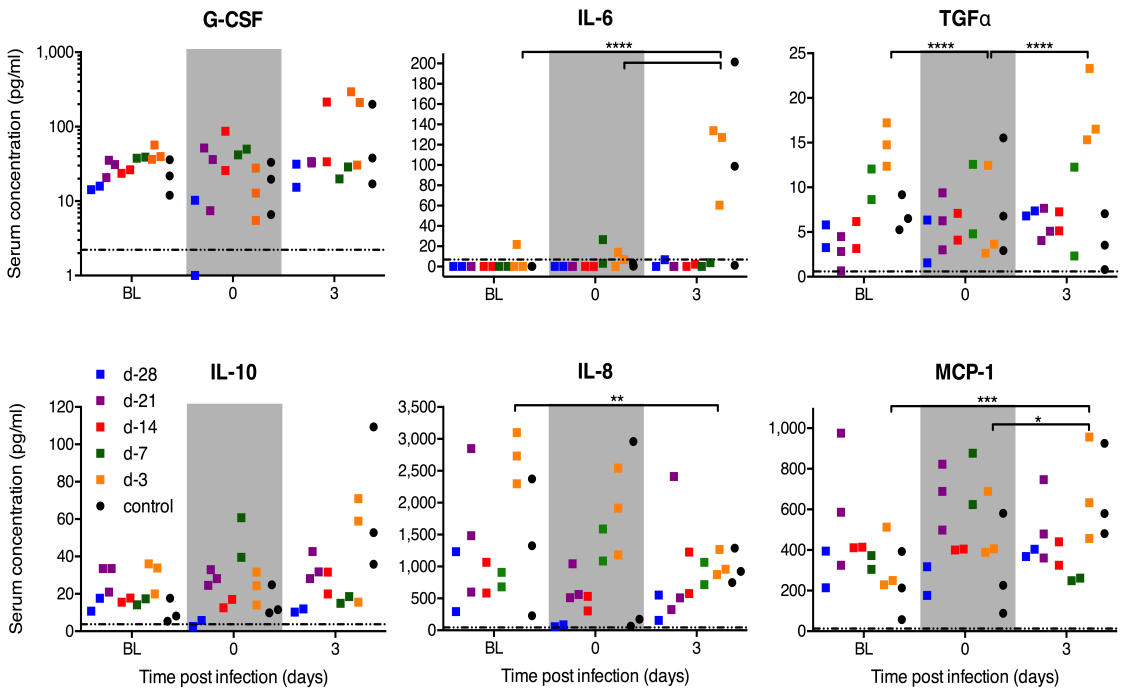

Fig. S2: Serum cytokine levels of NHPs. Serum concentrations for GCS-F, IL-6, TGFα, IL-10, IL-8, and MCP-1 were determined prior to vaccination (base line, BL), on the day of challenge (day 0) and early after EBOV-Makona challenge (day 3). Each square represents an individual animal at the indicated time point. The dotted line in each panel marks the assay detection limit. Two-way ANOVA with Tukey multiple comparison post test was used to analyze statistical significance for the day -3 vaccination group at different time points. Significance levels are indicated as follows: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****).