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LETTER

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Recombinant Sudan virus and evaluation of humoral cross-reactivity between Ebola and Sudan virus glycoproteins after infection or rVSV-ΔG-ZEBOV-GP vaccination

Markus H. Kainulainen, Jessica R. Harmon, Amy N. Whitesell, Éric Bergeron, Elif Karaaslan, Caitlin M. Cossaboom, Jason H. Malenfant, Aaron Kofman, Joel M. Montgomery, Mary J. Choi, César G. Albariño and Christina F. Spiropoulou

Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, USA

ABSTRACT
Ebola disease outbreaks are major public health events because of human-to-human transmission and high mortality. These outbreaks are most often caused by Ebola virus, but at least three related viruses can also cause the disease. In 2022, Sudan virus re-emerged causing more than 160 confirmed and probable cases. This report describes generation of a recombinant Sudan virus and demonstrates its utility by quantifying antibody cross-reactivity between Ebola and Sudan virus glycoproteins after human infection or vaccination with a licensed Ebola virus vaccine.

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KEYWORDS Sudan virus; recombinant virus; Ebola vaccine; cross-protection; neutralizing antibodies

Main text

Four members of the genus *Orthoebolavirus* (family *Filoviridae*) – Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus, and Taï Forest virus – are capable of causing Ebola disease, a condition typically progressing from non-specific febrile illness to severe gastrointestinal symptoms, coagulopathies, and multi-organ dysfunction with high mortality. Ebola disease outbreaks usually begin as zoonotic spillovers from animal reservoir hosts not definitively identified and expand via human-to-human contact transmission [1]. Although EBOV is the most frequent cause of Ebola disease outbreaks and historically the filovirus garnering the most research interest, SUDV has also emerged repeatedly and proven capable of causing expanding outbreaks with high mortality, most recently in 2022 [2,3]. Here, we generate a recombinant SUDV to address neglected research needs and demonstrate its utility by quantifying neutralization specificity between EBOV and SUDV after infection with either virus or vaccination with the EBOV vaccine, rVSV-ΔG-ZEBOV-GP.

Assays with infectious viruses, particularly those with modest cytopathic effect, benefit from easy visualization of infected cells. A common approach to circumvent immunostaining is to add a reporter gene into the viral genome so that fluorescence can be directly seen under the microscope or quantified using an automated microplate reader. To facilitate high-throughput assays with authentic SUDV (variant Gulu), we rescued a recombinant virus that expresses the fluorescent reporter ZsGreen (ZSG) at the N-terminus of viral protein 40 (VP40). Separation of ZSG and VP40 is achieved by the P2A peptide [4], an insertion strategy shown to work well in rescue of EBOV [5]. In short, viral antigenomic RNA is produced from a rescue plasmid by transfected T7 RNA polymerase and replication is initiated by EBOV RNA-dependent RNA polymerase (L), nucleoprotein (NP), and VP30 and VP35 proteins provided in trans (Supplementary Materials and Methods, Supplementary Table 2).

The recombinant virus, rSUDV-ZSG, produced robust fluorescence signal in Vero-E6 and Huh7 cells and grew with similar kinetics as the corresponding isolate virus, reaching only marginally lower maximal titres (Figure 1A,B). Interestingly, although we could produce a virus stock with the intended consensus sequence, repeated successful rescue experiments indicated that a region near the C-terminus of the viral nucleoprotein (NP) was under mutational

CONTACT Christina F. Spiropoulou ccs8@cdc.gov Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, CDC/H18-SB/SB153, 1600 Clifton Rd, Atlanta, GA 30329, USA
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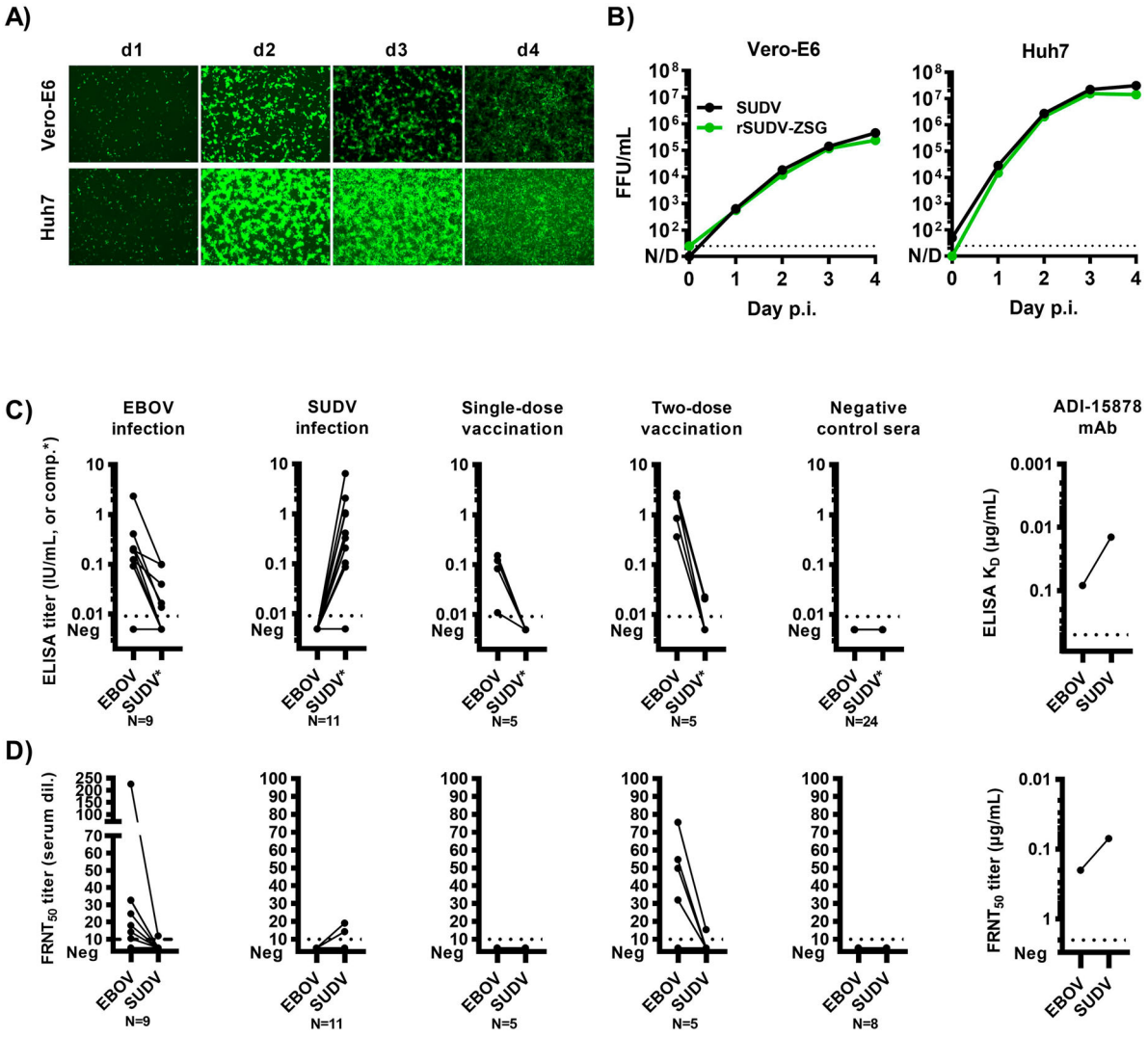

Figure 1. Recombinant SUDV expressing the reporter gene ZsGreen, and humoral cross-reactivity between EBOV and SUDV glycoproteins after infection and vaccination. Spread of rSUDV-ZSG in Vero-E6 and Huh7 monolayers (A). Multi-cycle growth curves of rSUDV-ZSG and isolate SUDV variant Gulu in the respective cell lines (multiplicity of infection = 0.2) (B). Serum samples collected after EBOV or SUDV infection or vaccination with rVSV-ΔG-ZEBOV-GP were analysed by ELISA to determine IgG antibody titres against EBOV GP as international units/mL, or as arbitrary fold-units against SUDV GP for comparison purposes. For known cross-reactive mAb ADI-15878 [6], the equilibrium dissociation constant K_D was determined from ELISA binding curves (C). Neutralization of EBOV and SUDV reporter viruses (D). FFU, focus-forming unit; IU, international units; $FRNT_{50}$, focus-reduction neutralization titre 50%.

pressure (Supplementary Table 1). Insertion of the reporter gene itself was found to be stable with ≥99% of virus foci expressing ZSG at passage 5.

The recent outbreak caused by SUDV highlighted the fact that vaccines are only available for Ebola disease caused by EBOV. To quantify any cross-reactivity between SUDV and EBOV, we measured antibodies binding stabilized [7] SUDV and EBOV structural glycoproteins in samples from humans infected with either virus or vaccinated against EBOV with 1 or 2 doses of the rVSV-ΔG-ZEBOV-GP vaccine. Five out of nine survivors of EBOV infection [8] had IgG antibodies that bound SUDV GP with 13-fold median titre reduction (range 2–30-fold). Conversely, no IgG binding EBOV GP were found after SUDV infection ($N = 11$). No antibodies against SUDV GP were detected after a single dose of the rVSV-ΔG-ZEBOV-GP vaccine in 5 donor sera, while 2 out of 5 boosted individuals had such antibodies, with 110–118-fold titre reduction. Neutralization assays using rSUDV-ZSG and a previously reported recombinant EBOV-GFP [9] showed that neutralization activity, when present, was largely specific to the cognate virus. One out of nine EBOV infection survivors and one out of five boosted vaccinees demonstrated cross-reactive neutralization, with 19-fold and 5-fold titre reductions, respectively.

Discussion

This work describes the generation of a recombinant SUDV carrying a fluorescent reporter gene. Interestingly, the rescue experiments demonstrated genetic instability near the C-terminus of the viral

nucleoprotein (NP), including position 711. Intriguingly, NP R711C was one of the most prominent mutations accumulated by SUDV variant Mubende during the 2022 outbreak [10]. These observations imply that this region is a hotspot for adaptation in cell culture and possibly also in humans.

EBOV is currently the only filovirus against which there are licensed vaccines. Out of these, rVSV-ΔG-ZEBOV-GP has demonstrated efficacy in the context of an outbreak [11]. Although some cross-protection against SUDV was observed in guinea pigs [12], vaccinated and EBOV-challenged non-human primates were not protected against subsequent SUDV challenge [13,14]. Indeed, the vaccine is indicated for protection against EBOV only (EMA/CHMP/557387/2019 and FDA BL 125690/0). Knowing that in non-human primates the elicited protection against EBOV depends on antibodies (but not $CD8^+$ T cells) [15] we quantified the humoral cross-reactivity in humans. We find that antibody cross-reactivity between EBOV and SUDV structural glycoproteins occurs only occasionally and typically with markedly reduced binding and neutralization titres. Together with the animal model data, our findings support the conclusion that specific vaccines against SUDV should be developed to prepare for future outbreaks.

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Disclaimer

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.

Disclosure statement

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ORCID

Markus H. Kainulainen http://orcid.org/0000-0003-2492-1210

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