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SUPPLEMENT ARTICLE

Passive Immunotherapy: Assessment of Convalescent Serum Against Ebola Virus Makona Infection in Nonhuman Primates

Chad E. Mire,[1,2] Joan B. Geisbert,[1,2] Krystle N. Agans,[1,2] Emily P. Thi,[3] Amy C.H. Lee,[3] Karla A. Fenton,[1,2] and Thomas W. Geisbert[1,2]

[1]Galveston National Laboratory, and [2]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston; and [3]Arbutus Biopharma, Burnaby, Canada

Background. Convalescent serum and blood were used to treat patients during outbreaks of *Zaire ebolavirus* (ZEBOV) infection in 1976 and 1995, with inconclusive results. During the recent 2013–2016 West African epidemic, serum/plasma from survivors of ZEBOV infection was used to treat patients in the affected countries and several repatriated patients. The effectiveness of this strategy remains unknown.

Methods. Nine rhesus monkeys were experimentally infected with ZEBOV-Makona. Beginning on day 3 after exposure (at the onset of viremia), 4 animals were treated with homologous ZEBOV-Makona convalescent macaque sera, 3 animals were treated in parallel with heterologous *Sudan ebolavirus* (SEBOV) convalescent macaque sera, and 2 animals served as positive controls and were not treated. Surviving animals received additional treatments on days 6 and 9.

Results. Both untreated control animals died on postinfection day 9. All 4 ZEBOV-Makona–infected macaques treated with homologous ZEBOV-Makona convalescent sera died on days 8–9. One macaque treated with heterologous SEBOV convalescent sera survived, while the other animals treated with the heterologous SEBOV sera died on days 7 and 9.

Conclusions. The findings suggest that convalescent sera alone is not sufficient for providing 100% protection against lethal ZEBOV infection when administered at the onset of viremia.

Keywords. Ebola virus; convalescent serum; immunotherapy; post exposure treatment.

The filoviruses Marburg virus and Ebola virus cause severe and often fatal hemorrhagic fever in humans and nonhuman primates (NHPs). The *Ebolavirus* (EBOV) genus is divided into 5 species: *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Tai Forest ebolavirus* (also known as *Côte d'Ivoire ebolavirus* or *Ivory Coast ebolavirus*), *Reston ebolavirus*, and *Bundibugyo ebolavirus* [1]. The ZEBOV epidemic in West Africa that spanned late 2013 to early 2016 has highlighted the need for effective, approved therapeutics and vaccines to respond to and/or prevent future outbreaks of this magnitude. Amazing progress has been made toward developing anti-ZEBOV glycoprotein (GP) monoclonal antibody cocktails as therapeutics [2–4], culminating in the most recent report detailing the successful treatment of NHPs with the antibody cocktail ZMapp at the onset of severe signs of ZEBOV-induced disease [5]. While this result was indeed groundbreaking for ZEBOV antibody therapy, a complete response to an outbreak with the scope of the West Africa ZEBOV-Makona variant by using this method of treatment would have required production levels that were not available at the time of the outbreak or at present. Considering the limited supply of experimental therapeutics at the peak of the epidemic, the World Health Organization (WHO) released guidance on the use of ZEBOV convalescent whole blood or plasma to respond to the epidemic because the affected West African countries had the resources to use these potential treatments [6]. In fact, clinical trials using convalescent blood–based products were initiated in Liberia, Sierra Leone, and Guinea [7–9]; the results of one of these trials revealed no benefit of convalescent plasma, although anti-ZEBOV neutralizing immunoglobulin levels were unknown [10]. This is not a new consideration for the treatment of EBOV infection, as there was limited use of convalescent serum in 1976 [11] and convalescent whole blood in 1995 [12] in patients infected with ZEBOV. Whether these treatments were successful is still unknown as there were many confounding factors, such as supportive care and timing of treatment given to patients.

In addition to EBOV infections, convalescent blood–based products have been used to treat human infections due to measles virus [13], Lassa virus [14], SARS coronavirus, and influenza A(H5N1) virus [15]. Passive antibody therapy for Lassa in particular was also examined in nonhuman primates (NHPs), and protection was shown to be dependent on lineage-specific neutralizing antibody [16]. While convalescent immunotherapy has proved beneficial for a number of viruses, the question

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Correspondence: T. W. Geisbert, University of Texas Medical Branch, Galveston National Laboratory, 301 University Blvd, Galveston, TX 77550-0610 (twgeisbe@utmb.edu).

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about its usefulness against filoviruses has remained unanswered. To address whether convalescent whole-blood treatment was efficacious against ZEBOV infection, as was suggested during the 1995 Kikwit outbreak [12], a previous study used convalescent whole blood to treat NHPs, with no observable beneficial effect [17], which was similar to results with high-titer anti-ZEBOV equine immunoglobulin treatment [18]. These results seemed to suggest that convalescent immunotherapy against ZEBOV was not useful. However, a subsequent study used purified immunoglobulin G (IgG) antibodies from vaccinated NHPs that survived ZEBOV challenge [2]. Unlike the previous studies in NHPs, postexposure treatment with these antibodies provided protection against ZEBOV, with mild clinical signs of illness observed.

Considering the use of convalescent immunotherapy during the most recent ZEBOV epidemic and the success of anti-ZEBOV monoclonal antibody therapy in NHPs, we were interested in examining whether convalescent sera (CS) obtained from NHPs 28 days after ZEBOV exposure could confer any protective benefit against ZEBOV-Makona infection. These CS would most likely represent a model in which survivors of an outbreak could donate serum once they tested negative for ZEBOV viremia by viral RNA diagnostic assays and contribute to containing an outbreak with a local resource. Here, we assessed the protective efficacy of CS from NHPs surviving ZEBOV-Makona challenge in a macaque model of ZEBOV infection.

MATERIALS AND METHODS

Anti-EBOV IgG Serum Analysis

EBOV RNA–negative sera collected at study end point from antiviral therapeutic ZEBOV-Makona or SEBOV-Gulu studies were tested for cross-reactive IgG antibodies against SEBOV and ZEBOV. Enzyme-linked immunosorbent assays (ELISAs) using recombinant ZEBOV GP (Integrated BioTherapies), recombinant SEBOV GP (Sino Biological), or purified virus-like particles (VLPs) containing nucleoprotein (NP), viral protein 40 (VP40), and GP antigen for the appropriate EBOV were used to detect cross-reactive IgG as previously described [19]. VLPs were produced as previously described [19].

Avidity measurement of total IgG to EBOV GP was determined using a displacement ELISA to establish the concentration of sodium thiocyanate (NaSCN) needed to dissociate 50% of the antibody-antigen interactions. Briefly, Immulon MaxiSorp flat-bottomed 96-well plates (catalog no. 12-565-136; Thermo Fisher) were coated for ≥18 hours at 4°C with 100 μL/well with either 0.1 μg/mL recombinant ZEBOV GP (Integrated BioTherapies), 0.5 μg/mL recombinant SEBOV GP (Sino Biological), SEBOV VP40/GP/NP VLPs, or ZEBOV VP40/GP/NP VLPs in carbonate/bicarbonate buffer. Plates were washed twice with 300 μL/well of 1× phosphate-buffered saline (PBS) and blocked for ≥2 hours at room temperature with 300 microliters/well of 1×PBS containing 10% fetal bovine serum (FBS). Plates were then washed 6 times with 300 microliters/well of 1×PBS containing 0.2% Tween-20. NHP serum samples were then assayed in duplicate, using a previously determined dilution (1:100 for ZEBOV-CS and 1:50 for SEBOV-CS) to produce an OD of 0.8 at 405 nm. Plates were incubated for 1 hour at room temperature and washed. Increasing concentrations of sodium thiocyanate diluted in PBS (0 M, 1 M, 2 M, 3 M, 4 M, 5 M, and 6 M) were used to treat wells (100 μL/well) for 15 minutes at room temperature. Plates were washed and then incubated with 100 μL/well anti-monkey IgG conjugated to horseradish peroxidase (HRP; Fitzgerald Industries) at a 1:2500 dilution for 1 hour at room temperature. Plates were washed and developed with 100 microliters/well of ABTS-1 C ELISA HRP substrate system (2,2′-azine-di[3-ethylbenzthiazoline-6-sulfonate]; Thermo Fisher). After ≤10 minutes, the reaction was stopped by adding 100 mL/well of 1% sodium dodecyl sulfate (Sigma). Absorbance was measured at 405 nm in an Emax plate reader (Molecular Devices, Sunnyvale, California).

Neutralizing antibody titers were determined by performing plaque reduction neutralization titration (PRNT) assays. Briefly, Vero cells were seeded into 6-well plates to generate a confluent monolayer on the day of infection. Serum dilutions were prepared in Dulbecco's modified Eagle's medium and 100 μL were incubated with approximately 100 plaque-forming units of ZEBOV-Makona in a total volume of 200 μL at 37°C for 60 minutes. Media was removed from cells, the serum-virus mixture was added in duplicate, and samples were incubated for 60 minutes at 37°C. The mixture was removed from the cells and 2 mL of 0.9% agarose in Eagle's minimum essential medium with 5% FBS was overlaid onto the wells. Cells were observed 7 days after incubation, and plaques were counted using neutral red stain. The neutralizing antibody titer of a serum sample was considered positive at a dilution showing a ≥50% reduction ($PRNT_{50}$), compared with the virus control without serum.

Animal Challenge

Nine healthy adult rhesus macaques (*Macaca mulatta*) of Chinese origin (weight, 4–8 kg) were inoculated intramuscularly with 1000 plaque-forming units (PFU) of ZEBOV-Makona [20, 21]. Either ZEBOV-Makona (n = 4) or SEBOV-Gulu (n = 3) CS (pooled from survivors; ZEBOV-CS was from 6, and SEBOV-CS was from 3) were administered by bolus intravenous infusion (approximately 10 mL) and bolus subcutaneous administration (approximately 60 mL) 3 days after ZEBOV-Makona challenge, while the control animals (n = 2) were not treated. All serum used was administered as a total of approximately 70 mL, split 10 mL and 60 mL for intravenous and subcutaneous administration; this is roughly equivalent to approximately 11 mL/kg, which is twice as much volume than used to treat repatriated ZEBOV-Makona–infected patients [22]. Surviving treated animals received additional treatments of convalescent sera on days 6 and 9 after ZEBOV-Makona

challenge. All animals were given physical examinations, and blood was collected at the time of challenge and on days 3, 6, 9, 14, 21, and 28 after ZEBOV-Makona challenge. In addition, all animals were monitored daily and scored for disease progression with an internal filovirus scoring protocol approved by the University of Texas Medical Branch–Galveston (UTMB) Institutional Animal Care and Use Committee. The scoring changes measured from baseline included posture/activity level, attitude/behavior, food and water intake, weight, respiration, and disease manifestations, such as visible rash, hemorrhage, ecchymosis, or flushed skin; scores for each of these conditions increase as severity increases. A score of ≥9 indicated that an animal met criteria for euthanasia. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and it adhered to principles stated in the eighth edition of the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. The facility where this research was conducted (UTMB) is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and has an approved Office of Laboratory Animal Welfare Assurance (assurance no. A3314-01).

Conducting animal studies with NHPs in a biosafety level 4 (BSL-4) facility severely restricts the number of animal subjects, the volume of biological samples that can be obtained, and the ability to repeat assays independently and thus limits statistical analysis. Consequently, data are presented as the mean calculated from replicate samples, not replicate assays, and error bars represent the standard deviation across replicates. With the small numbers of animals used, 100% survival of treated animals is required for survival significance to be attained.

Detection of Viremia

RNA was isolated from whole blood by means of the Viral RNA mini-kit (Qiagen), placing 100 μL of blood into 600 μL of buffer AVL. Primers/probe targeting the VP30 gene of ZEBOV were used for real-time quantitative polymerase chain reaction (qPCR) with the probe used here being 6-carboxyfluorescein–5′CCG TCA ATC AAG GAG CGC CTC 3′–6 carboxytetramethylrhodamine (Life Technologies). ZEBOV RNA was detected using the CFX96 detection system (BioRad Laboratories) in One-step probe real-time qPCR kits (Qiagen) as previously described [21]. The limit of detection (LOD) for the real-time qPCR is 1×10^4 genome equivalents per gram of tissue. Virus titration was performed by a plaque assay with Vero E6 cells from all serum samples as previously described [23]. Briefly, increasing 10-fold dilutions of the samples were adsorbed to Vero E6 monolayers in duplicate wells (200 μL); the limit of detection was 5 PFU/mL.

Hematologic and Serum Biochemical Analyses

Total white blood cell counts, white blood cell differentials, red blood cell counts, platelet counts, hematocrit values, total hemoglobin concentrations, mean cell volumes, mean corpuscular volumes, and mean corpuscular hemoglobin concentrations were analyzed using a laser-based hematologic analyzer (Beckman Coulter) in blood collected in tubes containing ethylenediaminetetraacetic acid. Serum samples were tested for concentrations of albumin, amylase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, glucose, cholesterol, total protein, total bilirubin, blood urea nitrogen, creatine, and C-reactive protein by using a Piccolo point-of-care analyzer and Biochemistry Panel Plus analyzer discs (Abaxis).

Histopathologic and Immunohistochemical Analyses

Necropsy was performed on all subjects. Tissue samples of all major organs were collected for histopathologic and immunohistochemical examination, immersion-fixed in 10% neutral buffered formalin, and processed for histopathologic analysis as previously described [21].

RESULTS

Analysis of EBOV Convalescent Sera

To test whether early convalescent sera is useful against ZEBOV-Makona infection, we pooled PCR-negative serum from several NHPs that survived either ZEBOV-Makona or SEBOV-Gulu infection. The SEBOV-Gulu convalescent sera were used as an EBOV species control to investigate whether species-specific serum was necessary for treatment. While it is possible that convalescent serum can contain factors other than specific antibodies necessary for therapeutic benefit, we focused on the antibody aspect and assessed both sera for the level of IgG to the following ZEBOV or SEBOV proteins: GP alone or GP, NP, and VP40 in VLPs by ELISA. High titers of IgG that could bind to SEBOV GP were detected in the SEBOV-Gulu sera as compared to minimal levels bound to ZEBOV GP and both VLPs (Figure 1*A*). Similar levels of IgG that could bind SEBOV and ZEBOV GP were detected in the ZEBOV-Makona convalescent sera, whereas the levels of IgG bound to ZEBOV VLPs were high, with levels bound to SEBOV VLPs minimal (Figure 1*A*). While some cross-reactivity was observed in both sets of sera, each set had higher levels of IgG, indicative of convalescence from each particular species of EBOV; we detected no variation in the IgG titers for the antigens between individual donors, except for the ZEBOV-CS to ZEBOV VLP. Next, the ability of ZEBOV-Makona and SEBOV-Gulu convalescent sera to neutralize ZEBOV-Makona was tested by a $PRNT_{50}$ assay. Only the ZEBOV-Makona convalescent sera were able to modestly neutralize ZEBOV-Makona in vitro, with a $PRNT_{50}$ of 1:40 (Figure 1*B*). The IgGs in each convalescent sera were further tested for their quality by an avidity assay, using ZEBOV VLPs; there was no appreciable difference between the sera (Figure 1*C*).

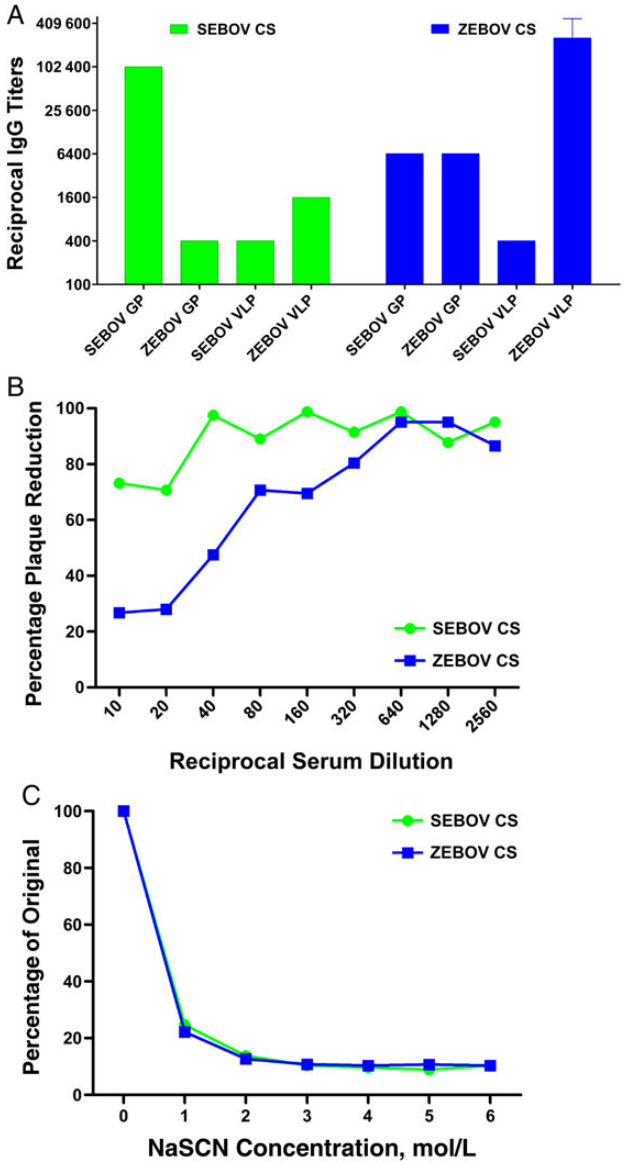

Figure 1. *A*, Reciprocal end point dilution titers for immunoglobulin G (IgG) from *Sudan ebolavirus* (SEBOV) convalescent sera (CS; green) or *Zaire ebolavirus* (ZEBOV) CS (blue) against SEBOV glycoprotein (GP), ZEBOV GP, SEBOV virus-like particles (VLPs; containing nucleoprotein [NP], viral protein 40 [VP40], and GP antigens), and ZEBOV VLPs (containing NP, VP40, and GP antigens). *B*, Plaque reduction neutralization assay for SEBOV CS (green) or ZEBOV CS (blue) against ZEBOV-Makona infection. *C*, Avidity assay for binding of IgG from SEBOV CS (green) or ZEBOV CS (blue) against ZEBOV VLPs (containing NP, VP40, and GP antigens) in the presence of increasing concentration of sodium thiocyanate (NaSCN). Error bars represent standard deviations.

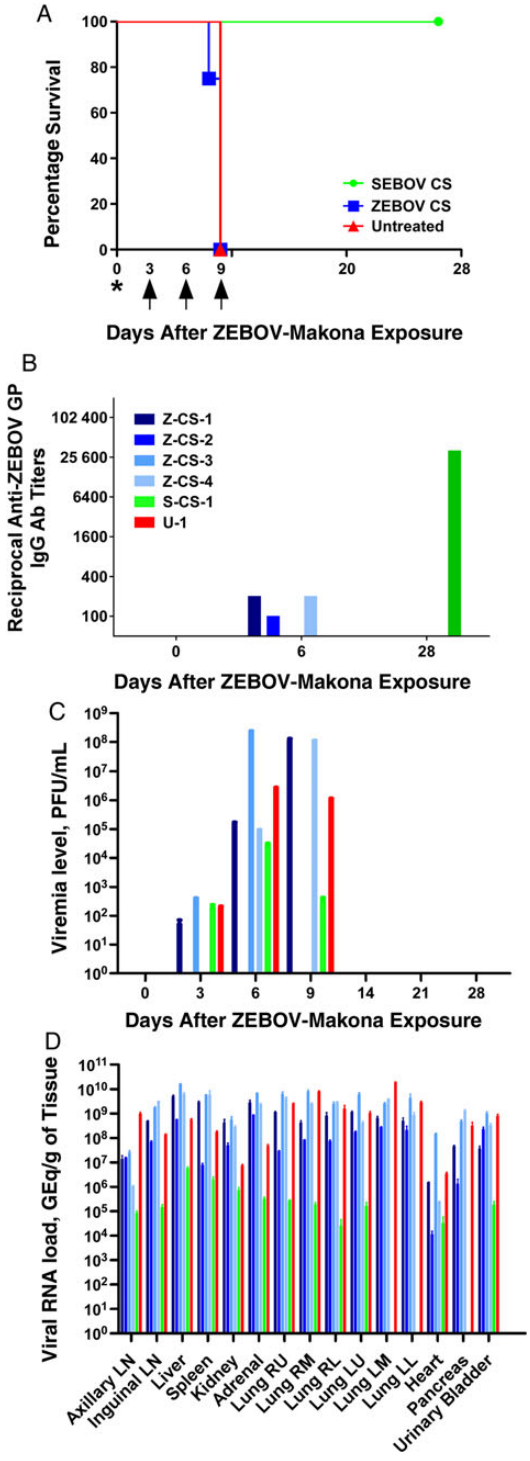

Figure 2. *A*, Kaplan–Meier survival curve for animals after *Zaire ebolavirus* (ZEBOV)–Makona exposure treated with *Sudan ebolavirus* (SEBOV) convalescent sera (CS; green: n = 1), with ZEBOV CS (blue; n = 4), or untreated (red; n = 1). The asterisk indicates the day of ZEBOV-Makona exposure, and the arrows indicate the days of CS treatment. *B*, Reciprocal end point dilution titers for anti-ZEBOV glycoprotein (GP) immunoglobulin G (IgG) for animals treated with SEBOV CS (green), with ZEBOV CS (blue), or untreated (red). *C*, Infectious viremia level for animals treated with SEBOV CS (green), with ZEBOV CS (blue), or untreated (red). *D*, Viral RNA load for animals treated with SEBOV CS (green), with ZEBOV CS (blue), or untreated (red). Abbreviations: Ab, antibody; GEq, genome equivalents; LL, left lower; LM, left middle; LN, lymph node; LU, left upper; PFU, plaque-forming units; RL, right lower; RM, right middle; RU, right upper.

Evaluation of Convalescent Sera Against ZEBOV-Makona Infection

After detecting anti-ZEBOV IgG and observing neutralization of ZEBOV-Makona in vitro from the ZEBOV-Makona convalescent sera, we assessed a regimen of 3 total convalescent sera treatments once every 3 days beginning on day 3 after exposure (Figure 2*A*) in a ZEBOV-Makona NHP model of infection. Six rhesus macaques were infected intramuscularly with 1000 PFU

of ZEBOV-Makona (Figure 2*A*) and were either untreated (n = 1), treated with SEBOV-Gulu convalescent sera (n = 1), or treated with ZEBOV-Makona convalescent sera (n = 4). All animals were observed for clinical signs of disease and hematologic and blood chemical changes after ZEBOV-Makona exposure. Interestingly, the SEBOV-Gulu convalescent sera–treated animal (S-CS-1) survived infection, while the untreated control and all 4 ZEBOV-Makona convalescent sera-treated animals died of infection (Figure 2*A*). While animal S-CS-1 survived, it had a fever on day 6 after exposure, anorexia on days 6–11, and mild depression on day 10. However, this animal did not have a petechial rash or moderate-to-severe depression like all animals in the untreated and ZEBOV-Makona convalescent sera groups.

To determine whether treatment with convalescent sera led to circulating anti-ZEBOV GP IgG with some longevity, we performed an ELISA on serum samples obtained on day 6 after exposure. Circulating anti-ZEBOV GP IgG was detected on day 6 postexposure in the cohort treated with ZEBOV-Makona convalescent sera (Figure 2*B*). However, this circulating anti-ZEBOV GP IgG was unable to reduce the infectious viral load (Figure 2*C*) and viral RNA load in tissues (Figure 2*D*) of animals treated with ZEBOV-Makona convalescent sera, whereas the infectious viral load was reduced below levels of detection beyond day 9 for S-CS-1 (Figure 2*C*). Further analysis of the blood samples revealed changes in liver-associated enzymes (Figure 3*A* and 3*B*) and hematologic changes (Figure 3*C* and 3*D*) consistent with ZEBOV infection for all animals, with only S-CS-1 resolving these changes. S-CS-1 was also the only animal to be devoid of typical gross pathologic and histopathologic findings and of labeling of ZEBOV antigen by immunohistochemical stain (data not shown).

IgG Avidity for ZEBOV GP

The survival of animal S-CS-1 raised questions regarding possible differences between the SEBOV-Gulu and ZEBOV-Makona convalescent sera. Considering the data on ZMapp, which targets the ZEBOV GP [5, 24], we decided to further analyze the avidity of the IgG for the ZEBOV GP only, instead of in combination with NP and VP40 in VLPs as done previously (Figure 1*C*). To investigate the avidity of each convalescent sera to ZEBOV GP, an avidity assay was performed, and intriguingly a slight shift in binding ability of the SEBOV convalescent sera was observed for the pooled donor sera when compared to the pooled ZEBOV convalescent sera (Figure 4*A*). This shift led us to analyze the avidity of the anti-ZEBOV GP antibodies from each individual donor serum from either donor group. The avidity of each SEBOV serum donor was shifted, showing a similar trend when compared to the ZEBOV serum donor group as compared to the combined donor data. One SEBOV serum

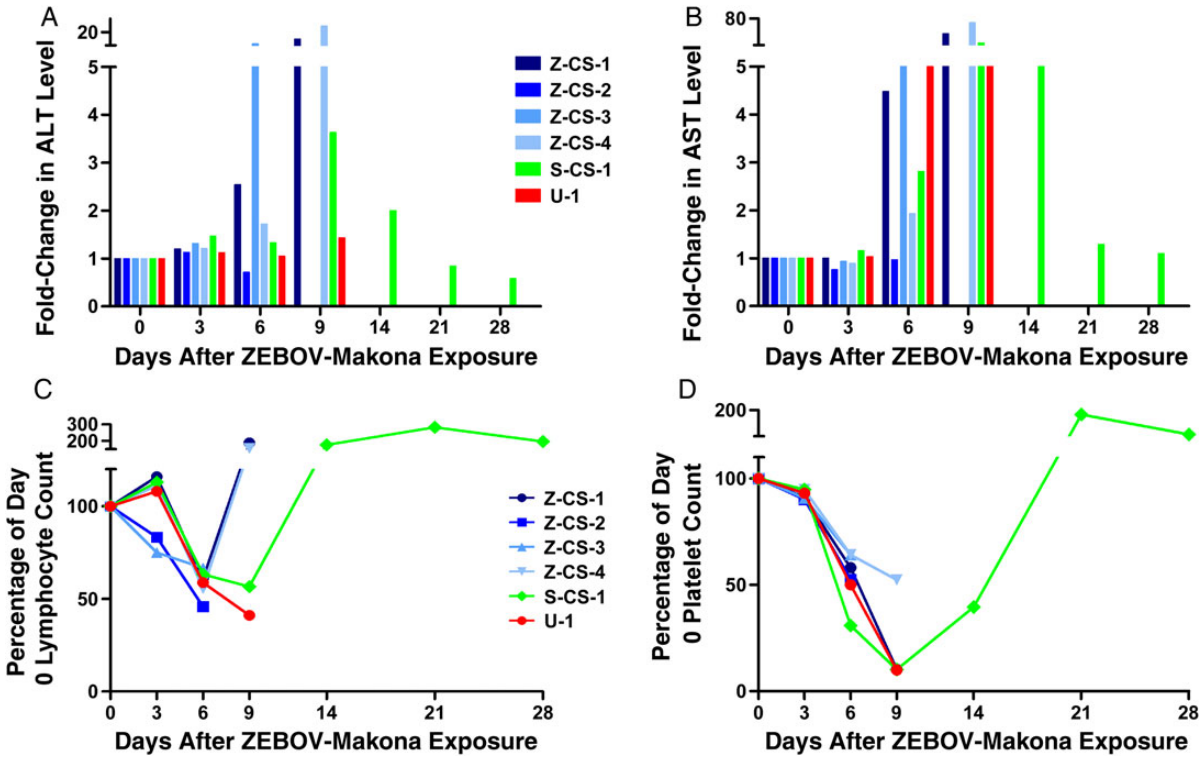

Figure 3. Circulating liver enzyme levels of alanine aminotransferase (ALT; *A*) and aspartate aminotransferase (AST; *B*) and circulating percentage baseline counts for lymphocytes (*C*) and platelets (*D*) for animals after *Zaire ebolavirus* (ZEBOV)–Makona exposure treated with *Sudan ebolavirus* (SEBOV) convalescent sera (CS; green), with ZEBOV CS (blue), or untreated (red).

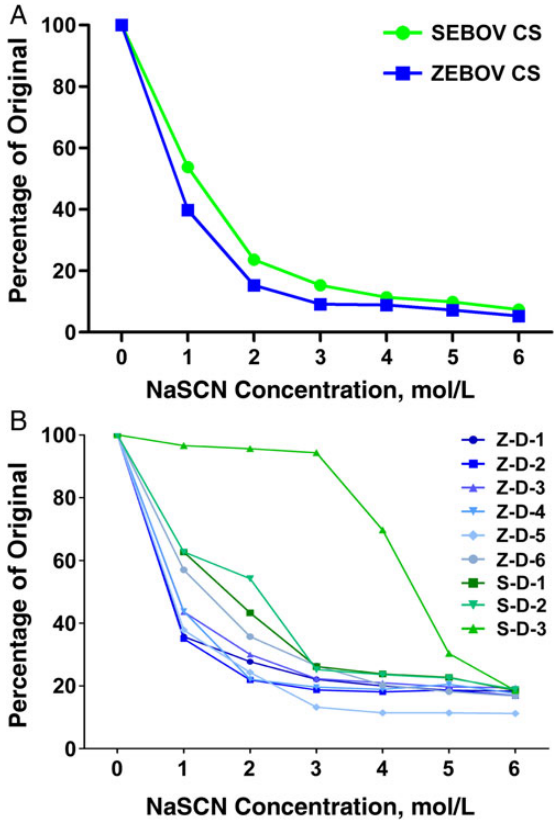

Figure 4. *A,* Avidity assay for binding of immunoglobulin G (IgG) from pooled *Sudan ebolavirus* (SEBOV) convalescent sera (CS; green) or pooled *Zaire ebolavirus* (ZEBOV) CS (blue) against ZEBOV GP in the presence of increasing concentration of sodium thiocyanate (NaSCN). *B,* Avidity assay for binding of immunoglobulin G from individual donors (D) of SEBOV CS (green) or ZEBOV CS (blue) against ZEBOV glycoprotein in the presence of increasing concentration of sodium thiocyanate (NaSCN).

donor in particular, S-D-3, had a very marked shift in the avidity assay (Figure 4*B*).

Further Evaluation of SEBOV Convalescent Sera Against ZEBOV-Makona Infection

The higher shift in avidity of the anti-ZEBOV GP IgG in the SEBOV convalescent sera suggested that the quality of antibody might be important for the protective effect observed in the lone NHP treated with these sera (Figure 2*A*). To assess whether the SEBOV convalescent sera was indeed protective, we assessed a regimen of 3 SEBOV-Gulu convalescent sera treatments once every 3 days, beginning on day 3 after exposure, in a ZEBOV-Makona NHP model of infection. Three NHPs were infected intramuscularly with 1000 PFU of ZEBOV-Makona and were with either untreated (n = 1) or treated with SEBOV-Gulu convalescent sera from the previous study (n = 1; S-CS-2) or a different SEBOV-Gulu convalescent sera pool (n = 1; S-CS-3). Unlike the previous observation, the SEBOV-Gulu convalescent sera–treated animals in this study did not survive infection, dying on days 7 (S-CS-2) and 9 (S-CS-3) after exposure, respectively, with the untreated control dying on day 9 after exposure, consistent with controls on the initial study (Figure 5*A*). All 3 animals had petechial rashes and moderate-to-severe depression with infectious viremia (Figure 5*B*), changes in liver-associated enzyme levels (Figure 5*C*), and thrombocytopenia (Figure 5*D*) consistent with ZEBOV-Makona infection.

DISCUSSION

In late 2014, the WHO released a report supporting the use of experimental treatments for evaluation of their effectiveness against ZEBOV-Makona infection and disease [6]. Convalescent whole-blood or plasma infusions were 2 of the treatments supported for use in clinical trials. Some of this support undoubtedly stemmed from the report of convalescent whole-blood treatment in the 1995 outbreak of ZEBOV-Kikwit infection, in which 7 of 8 treated patients survived [12], although the authors do not claim that the convalescent whole-blood treatment was efficacious. Additionally, another group reported that the likelihood of survival of these 7 treated patients was similar to the survival rate observed for the entire outbreak, as the treatments were initiated at later times after disease onset that were correlated with survival [25]. While these 7 patients survived ZEBOV-Kikwit infection and were treated with convalescent whole blood, the patients were essentially past the stage associated with morbid outcome when treatment occurred, so no therapeutic benefit of the convalescent whole blood could be determined. These observations, however, led to renewed interest in controlled studies involving passive immunotherapy in NHP models of ZEBOV infection. In particular, one study used convalescent whole blood to treat NHPs with no observable beneficial effect [17]. The convalescent whole blood used in this study was obtained from NHPs rechallenged with ZEBOV several years after initial exposure, and therefore the blood may not have had similar characteristics to convalescent whole blood collected at a time closer to the initial exposure. In the current study, we examined the protective effect of convalescent sera obtained about a month after initial ZEBOV exposure, which is consistent with conditions during the recent West African ZEBOV epidemic. In addition, we compared the efficacy of homologous ZEBOV-Makona–specific sera against heterologous SEBOV sera.

Surprisingly, our initial study resulted in survival for the heterologous SEBOV species convalescent sera–treated animal, while the NHPs treated with species-specific sera died of ZEBOV-Makona infection. While the SEBOV-Gulu convalescent sera had some cross-reactivity to ZEBOV GP, the titer for SEBOV GP in these sera was markedly higher. However, there was cross-reactivity, and with the success of anti-ZEBOV GP IgG treatments [3, 4], which was followed by the development of ZMapp [5], we analyzed whether the sera used herein had differences in the quality of IgG antibody to ZEBOV GP, using an avidity assay. Interestingly, we found that the SEBOV-Gulu convalescent sera had better-quality

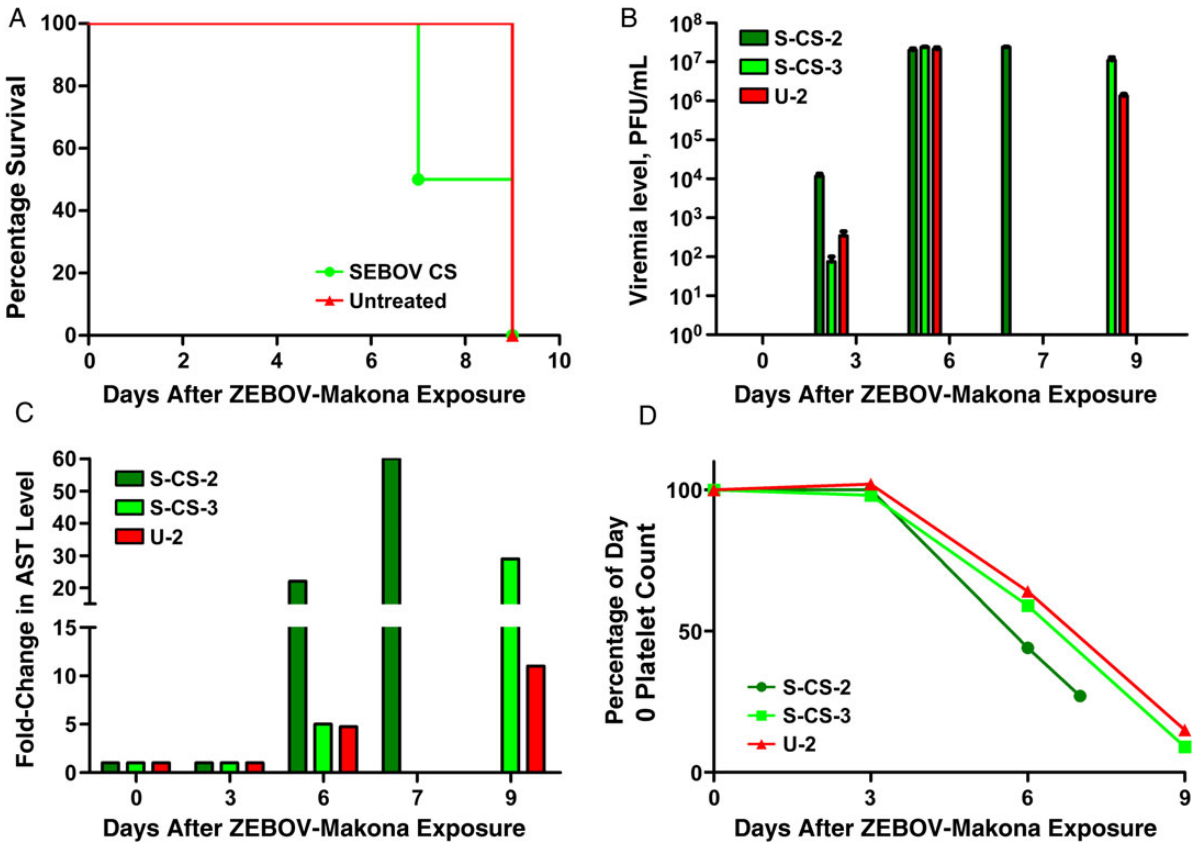

Figure 5. *A*, Kaplan–Meier survival curve for animals after *Zaire ebolavirus* (ZEBOV)–Makona exposure treated with *Sudan ebolavirus* (SEBOV) convalescent sera (CS; green; n = 2) or untreated (red; n = 1). *B*, Infectious viremia level for animals treated with SEBOV CS (green) or untreated (red). *C*, Circulating liver enzyme levels of aspartate aminotransferase (AST) for animals after ZEBOV-Makona exposure treated with SEBOV CS (green) or untreated (red). *D*, Circulating percentage of baseline counts for platelets for each group after ZEBOV-Makona exposure treated with SEBOV CS (green) or untreated (red). Abbreviation: PFU, plaque-forming units.

anti-ZEBOV GP IgG, from one donor in particular, than the ZEBOV-Makona convalescent sera. We speculated that perhaps the differences in anti-ZEBOV GP avidity accounted for the survival benefit observed and subsequently followed this study up with another study analyzing this same SEBOV-Gulu convalescent sera, as well as a different pool of SEBOV-Gulu convalescent sera. However, in this follow-up study, the NHPs treated with heterologous SEBOV-Gulu sera did not survive ZEBOV-Makona infection. These data suggest that the survivor in the initial study (Figure 2*A*) may not have received any protective benefit from the SEBOV-Gulu convalescent sera, although a small benefit from the SEBOV-Gulu sera cannot be ruled out in this study either. Importantly, previous studies with the Kikwit variant of ZEBOV in rhesus monkeys showed that, in rare cases, untreated control animals survive challenge [26]. The number of untreated control animals in the newly developed ZEBOV-Makona rhesus monkey model is not high enough at this time to determine whether a very small percentage of untreated control animals can survive.

Here, we show that treatment with convalescent sera beginning at day 3 after ZEBOV-Makona exposure could not provide a 100% protective benefit. It should be noted that all animals were viremic either by infectious virus or viral RNA detection on the day of treatment, which represents a high bar for therapeutic protection for any candidate therapy. This delayed treatment schedule was chosen in part due to the success of ZMapp with initiation of treatment at days 3, 4, or 5 after ZEBOV-Kikwit exposure [5]. The goal of the current study was to test whether viremic NHPs could show reversion of ZEBOV infection when treated with convalescent sera. We conclude that convalescent sera treatment initiated in NHPs at the onset of viremia is not an effective therapeutic, particularly when compared to the recent successes of the small interfering RNA [21] and ZMapp [5] therapeutics at advanced stages of disease. However, it is possible that treatment with convalescent sera at times earlier after exposure and/or using a different treatment regimen may provide protective benefit. Additionally, we used sera in this study, whereas convalescent whole blood or plasma obtained closer to EBOV exposure, as used in the WHO clinical trials [10], may also have protective blood coagulation factors not found in serum. While we cannot conclude that the anti-ZEBOV GP IgG avidity differences in the convalescent sera

accounted for the lone survivor we observed, the question of whether only IgG titer, avidity, or a combination of both is sufficient for passive immunotherapy to provide protection is an intriguing one.

Notes

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Disclaimer. The opinions, interpretations, conclusions, and recommendations contained herein are those of the authors and are not necessarily endorsed by UTMB.

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