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SUPPLEMENT ARTICLE

Efficacy of Vesicular Stomatitis Virus–Ebola Virus Postexposure Treatment in Rhesus Macaques Infected With Ebola Virus Makona

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The Ebola virus (EBOV) epidemic in West Africa increased the focus on vaccine development against this hemorrhagic fever–causing pathogen, and as a consequence human clinical trials for a few selected platforms were accelerated. One of these vaccines is vesicular stomatitis virus (VSV)–EBOV, also known as rVSV-ZEBOV, a fast-acting vaccine against EBOV and so far the only vaccine with reported efficacy against EBOV infections in humans in phase III clinical trials. In this study, we analyzed the potential of VSV-EBOV for postexposure treatment of rhesus macaques infected with EBOV-Makona. We treated groups of animals with 1 dose of VSV-EBOV either in a single injection at 1 or 24 hours after EBOV exposure or with 2 injections, half the dose at each time point; 1 control group received the same dose of the VSV-based Marburg virus vaccine at both time points; another group remained untreated. Although all untreated animals succumbed to EBOV infection, 33%–67% of the animals in each treatment group survived the infection, including the group treated with the VSV-based Marburg virus vaccine. This result suggests that protection from postexposure vaccination may be antigen unspecific and due rather to an early activation of the innate immune system. In conclusion, VSV-EBOV remains a potent and fast-acting prophylactic vaccine but demonstrates only limited efficacy in postexposure treatment.

Keywords. Ebola virus; VSV-EBOV; postexposure treatment; rhesus macaque.

Ebola virus (EBOV) belongs to the family Filoviridae, a group of negative-stranded RNA viruses known to cause hemorrhagic fever in humans and nonhuman primates (NHPs) with high case fatality rates [1]. EBOV is the causative agent of the largest recorded human epidemic of Ebola hemorrhagic fever in West Africa, with almost 29 000 infections and >11 300 fatalities [2]. In the United States, filoviruses are classified as select agents (www.selectagents.gov) and tier 1 pathogens, and they are also required to be handled in maximum containment laboratories worldwide [1]. In spite of this obstacle, several vaccine approaches [3] and treatment strategies [4] had been successfully tested in well-established EBOV macaque models before the emergence of EBOV-Makona in West Africa in late 2013.

In the face of this epidemic, the most promising experimental countermeasure approaches were quickly moved through phase I–III clinical trials (www.ClinicalTrials.gov; www.pactr.org). Among these platforms was the recombinant live-attenuated vesicular stomatitis virus (VSV)–based vaccine, VSV-EBOV, also known as rVSV-ZEBOV, which had previously been shown to protect NHPs from lethal EBOV challenge following a single dose vaccination [5]. Recently, this vaccine was shown to be fast acting because it completely protected NHPs from disease when a single vaccination was administered only 1 week before EBOV infection [6]. Preliminary reports from phase I and II human clinical trials showed promising potential of VSV-EBOV with regard to safety and immunogenicity [7–9], and the vaccine was moved into phase III efficacy trials in West Africa. The ring vaccination trial in Guinea was the first to demonstrate efficacy against EBOV infection in humans within 10 days, again highlighting the fast-acting character of VSV-EBOV and its usefulness as an emergency vaccine [10].

The fast-acting potential of VSV-EBOV raised the possibility of postexposure treatment. A previous NHP study exploring VSV-EBOV in postexposure treatment demonstrated 50% survival when a single dose was administered 20–30 minutes after EBOV challenge [11]. This result led to the first application of VSV-EBOV in a laboratory worker, who received a single dose of the vaccine approximately 49 hours after EBOV exposure [12].

In the current study, we sought to further investigate the potential of VSV-EBOV for postexposure treatment of EBOV-Makona–infected NHPs. We compared treatment using the same dose of VSV-EBOV in a single injection given 1 or 24 hours after EBOV infection with a 2-injection treatment, giving half the dose at both 1 and 24 hours. A control group was given the VSV-based Marburg virus vaccine (VSV-MARV) vaccine. Similar to previously reported findings [11], VSV-EBOV provided partial protection from lethal EBOV challenge. In contrast, however, in our study VSV-MARV treatment also

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resulted in survival, suggesting that protection from lethal EBOV infection was driven by antigen-unspecific innate immune responses.

METHODS

Animal Ethics and Biosafety Statement

All animal work was performed in strict accordance with the Guide for the Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare, National Institutes of Health and the Animal Welfare Act, United States Department of Agriculture. Animal procedures were conducted under anesthesia by trained personnel under the supervision of veterinary staff. All efforts were made to promote the welfare and to minimize animal distress in accordance with recommendations from the Weatherall report for the use of non-human primates.

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). Animals were monitored at least twice daily. Animals were fed commercial monkey chow twice daily and water was available ad libitum; they also received a variety of treats and fruit as food enrichment. Environmental enrichment consisted of manipulanda, music, and television. Humane end-point criteria, specified and approved by the Institutional Animal Care and Use Committee, 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, National Institute of Allergy and Infectious Diseases, Hamilton, Montana, applying standard operating protocols approved by the Institutional Biosafety Committee.

Vaccine and Challenge Virus

Good manufacturing practices (GMP)-grade VSV-EBOV (rVSV-ZEBOV), expressing the glycoprotein (GP) of the EBOV-Kikwit strain, was manufactured by KBP under the ownership of the Public Health Agency of Canada and used to treat macaques with a dose of 2×10^7 plaque-forming units (PFUs). Non–GMP-grade VSV-MARV, expressing the GP of the MARV-Angola strain, was produced by concentrating and purifying tissue culture supernatant and used to treat macaques with a dose of 2×10^7 PFUs. EBOV-Makona (Guinea C07; passage 1) [13, 14] was propagated on Vero E6 cells (mycoplasma negative), titrated on these cells, and stored in liquid nitrogen. Deep sequencing confirmed that the phenotype was approximately 95% 7U, representing wild-type EBOV and not the tissue culture–adapted 8U phenotype [15].

Study Design

This study included 15 rhesus macaques (*Macaca mulatta*), 13 male and 2 female, 3–4 years old and 3–5 kg in weight. The study was not blinded, and macaques were randomly divided into 5 study groups (n = 3). All animals were challenged intramuscularly on day 0 with a lethal dose of 1000 PFUs of EBOV-Makona (1-mL inoculum; confirmed by back-titration) at 2 sites in the caudal right thigh. Single-injection VSV-EBOV treatment was given by intramuscular injection of 2×10^7 PFUs in 0.5 mL at 1 site in the caudal aspect of the left thigh at 1 or 24 hours after EBOV infection. Groups receiving VSV-EBOV or VSV-MARV at 1 and 24 hours after EBOV infection were injected intramuscularly with 1×10^7 PFUs in 0.5 mL at 1 site in the caudal left thigh each time.

Physical examinations and blood sampling were performed at the time of EBOV infection; 12 hours later; and on days 1, 2, 3, 6, 9, 14, 21, 28, 35, and 42; and at the time of euthanasia. The animals were observed at least twice daily for clinical signs of disease, according to an Institutional Animal Care and Use Committee approved scoring sheet. Scores (0–15) were assigned for general appearance; skin and fur; nose, mouth, eyes, and head; respiration; feces and urine; food intake; and locomotor activity. These scores were recorded on an observation sheet, and animals were euthanized and necropsies were performed by board-certified veterinary pathologists when their total score reached the critical number of 35, or when any of the following signs were observed: impaired ambulation preventing access to food or water, excessive weight loss, lack of mental and physical alertness, labored breathing, or prolonged inability to remain upright.

Virus Loads

To determine virus loads in macaque blood samples, Vero E6 cells (mycoplasma negative) were seeded in 48-well plates the day before titration. Whole-blood samples were thawed, and 10-fold serial dilutions were prepared. Medium was removed from cells, and triplicate wells were inoculated with each dilution. After 1 hour, Dulbecco's minimum essential medium supplemented with 2% fetal bovine serum, penicillin-streptomycin, L-glutamine was added, and cells were incubated at 37°C. Cells were monitored for cytopathic effect, and the 50% tissue culture infectious dose was calculated for each sample, using the Reed and Muench method [16].

Hematology and Serum Chemistry

Total white blood cell, lymphocyte, neutrophil, platelet, reticulocyte and red blood cell counts and hemoglobin, and hematocrit values were determined from ethylenediaminetetraacetic acid (EDTA)–blood with the IDEXX ProCyte DX analyzer (IDEXX Laboratories). Serum biochemistry results (albumin, aspartate aminotransferase, alanine aminortransferase, γ-glutamyltransferase, serum urea nitrogen, and creatinine values) were analyzed using the Piccolo Xpress Chemistry Analyzer and Piccolo General Chemistry 13 Panel discs (Abaxis).

Humoral Immune Responses

Rhesus macaque serum samples were inactivated by γ-irradiation (5 mrad) and removed from the maximum containment laboratory according to Institutional Biosafety Committee–

approved standard operating procedures. Enzyme-linked immunosorbent assay (ELISA) plates were coated with EBOV-GPΔTM or MARV-GPΔTM antigen (both IBT Bioservices) overnight at 4°C, as described elsewhere [17]. For VSV-specific ELISA, Vero E6 cells were infected with wild-type VSV. After 2 days, the virus particles were harvested from the cell supernatant and concentrated through a 20% sucrose cushion for 2 hours at 20 000 rpm and 4°C. The wild-type VSV pellet was resuspended in phosphate-buffered saline (PBS), particles were lysed by addition of Triton X-100 (0.1% final concentration), and a 1:1 000 dilution of this solution in PBS was used to coat ELISA plates overnight at 4°C. For all ELISAs, after 3 washes with PBS/0.05% Tween, serial 2– or 4–fold dilutions of the serum samples were incubated in duplicate for 1 hour at 37°C. After 3 washes with PBS/0.05% Tween, horseradish peroxidase–conjugated anti-monkey immunoglobulin (Ig) G (KPL) was added for 1 hour, followed by additional washes and a final addition of substrate (KPL).

Optical density values were measured after 30 minutes, and samples were deemed positive when the optical density value was higher than the mean plus 3 standard deviations for negative serum samples. The end-point dilution titer is shown. EBOV GP–specific IgM responses were assessed using a commercial ELISA kit (Alpha Diagnostics). Serum samples were assayed in duplicates in a 1:400 dilution, and EBOV GP–specific IgM concentrations were calculated according to the manufacturer's instructions.

Serum Cytokine Levels

Serum samples (γ-irradiated with a 5 mrad dose) were diluted 1:2 in serum matrix for analysis with Milliplex Non-Human Primate Magnetic Bead Panel, according to the manufacturer's instructions (Millipore Corporation). Interferon (IFN) γ and IL-15 concentrations were determined for all samples, using the Bio-Plex 200 system (BioRad Laboratories). Levels of IFN-α were determined using the Cynomolgus/Rhesus IFN Alpha ELISA Kit (PBL Assay Science), according to the manufacturer's instructions.

VSV Detection

Total RNA was isolated from 140 μL of EDTA-treated blood samples at the indicated time points, using the QIAmp viral Mini RNA kit (Qiagen). All quantitative real-time reverse-transcription polymerase chain reaction analysis was 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; and reverse primer, CGAGCCATTCGACCACATC.

Statistical Analyses

Two-way analysis of variance with Sidak posttest analysis were used to determine statistical significance at the level of ≤.05. The Mantel-Cox method was used to analyze statistical significance for survival at the level of ≤.05. All analyses were completed using Prism software (GraphPad, version 6.0).

RESULTS AND DISCUSSION

VSV-EBOV (expressing the EBOV-Mayinga GP) previously demonstrated 50% protection of rhesus macaques against EBOV-Kikwit challenge after treatment with a single dose of 2×10^7 PFUs 20–30 minutes after challenge [11]. Based on this knowledge, we hypothesized that the survival from EBOV-Makona challenge would improve using VSV-EBOV (expressing the EBOV-Kikwit GP) with a 2-injection treatment approach at 1 and 24 hours versus a single injection at 1 or 24 hours. It should be noted that Mayinga, Kikwit, and Makona are distinct EBOV strains isolated at different times and geographic locations in Africa. The strains display known serological cross-reactivity and also show cross-protection in vaccine studies [6, 18].

Five groups of 3 animals each were infected with 1000 PFUs of EBOV-Makona intramuscularly. At 1 hour after infection, we injected 2×10^7 PFUs of VSV-EBOV intramuscularly for the single-injection group (NHPs 1–3), and 1×10^7 PFUs of VSV-EBOV (NHPs 4–6) or 1×10^7 VSV-MARV of (NHPs 10–12) for the 2-injection groups. Then, 23 hours later, NHPs 4–6 and NHPs 10–12 received the second intramuscular dose, 1×10^7 PFUs VSV-EBOV or 1×10^7 VSV-MARV, respectively. The 24-hour single-dose treatment group (NHPs 7–9) received a single injection of 2×10^7 PFUs of VSV-EBOV intramuscularly at 24 hours after infection. The control group (controls 1–3) remained untreated.

VSV-specific RNA could be detected in all treated animals as early as 12 hours after VSV-EBOV or VSV-MARV administration and persisted at least until day 3 after EBOV infection (Figure 1*A*), findings similar to previously published postexposure data in NHPs [11] and humans [12, 19]. By day 6, no VSV-specific RNA was detectable in any animal (Figure 1*A*). All the NHPs started to show clinical signs and pathophysiological blood parameters consistent with an early stage of Ebola hemorrhagic fever, including ruffled fur, hunched posture, fever, rash, thrombocytopenia, and elevated liver enzyme levels at about day 5 after EBOV challenge, with EBOV viremia first detected on day 6 (Figure 1*B*; Table 1).

By day 10, all untreated control animals (controls 1–3) had to be euthanized, as did NHP 1 (treatment at 1 hour), NHPs 5 and 6 (treatment at 1 and 24 hours), NHP 9 (treatment at 24 hours), and NHP 12 (VSV-MARV) (Table 1), resulting in nonsignificant differences in survival among all groups (Figure 1*C*). NHP 3 (1 hour treatment group) had to be euthanized on day 28 after EBOV infection. This animal had survived the acute phase of the disease but developed neurological symptoms and pneumonia leading to death (separate manuscript in preparation). Overall, the treatment groups together showed a 50% survival rate (6 of 12 animals), consistent with findings of the previously reported postexposure VSV-EBOV study [11].

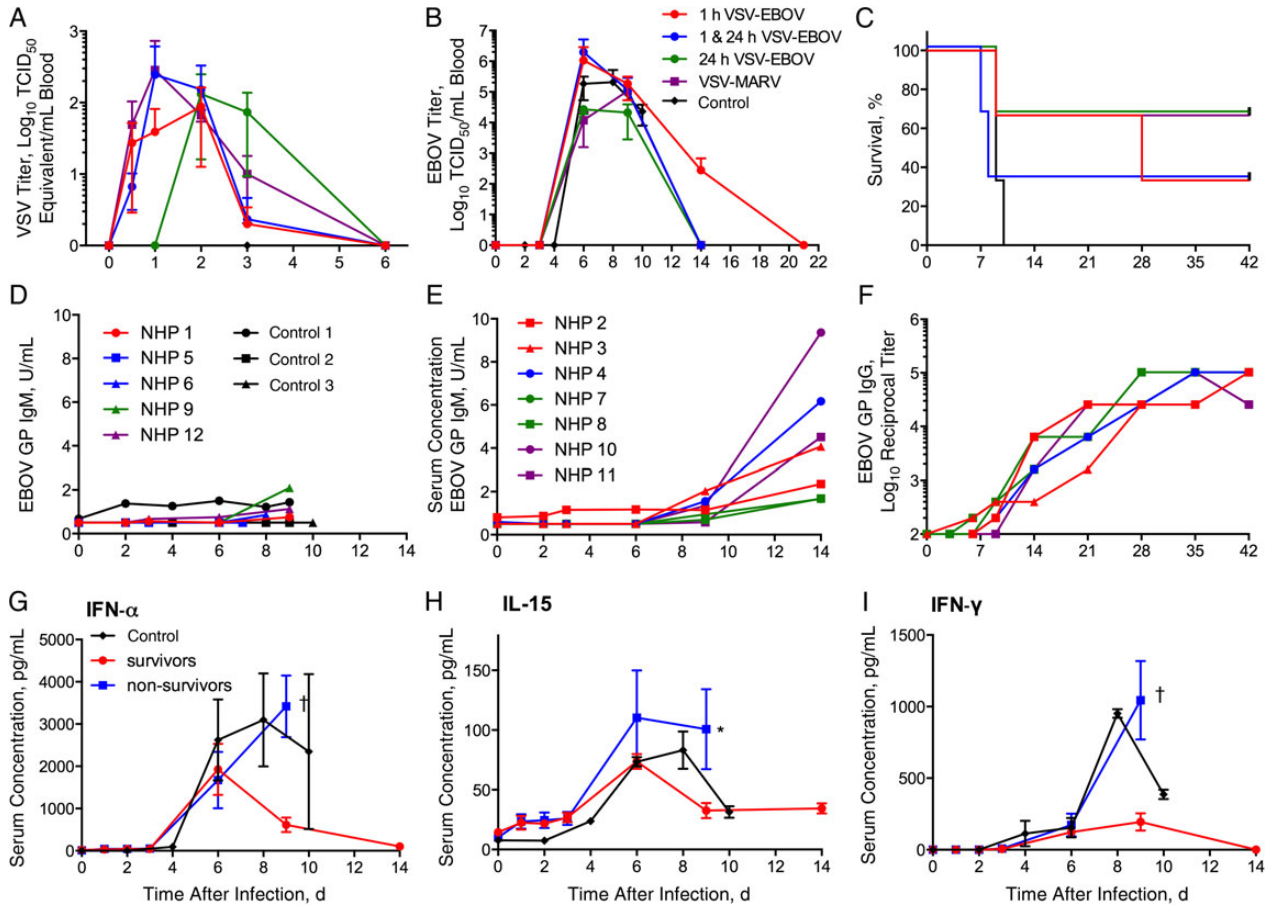

Figure 1. Viremia, survival, antibody titers and cytokine levels after Ebola virus (EBOV) infection. *A,* Vesicular stomatitis virus (VSV)–specific quantitative real-time reverse-transcription polymerase chain reaction was performed on RNA isolated from whole blood at the indicated time points; mean group values are shown with standard deviations (SDs). See key in *B. B,* EBOV titers in whole blood collected at the indicated time points; mean group titers are shown with SDs. *C,* Survival curves; see key in *B. D,* EBOV glycoprotein (GP)–specific immunoglobulin (Ig) M levels in the serum of animals succumbing to infection. *E,* EBOV GP–specific IgM levels in the serum of animals surviving infection. *F,* EBOV GP–specific IgG titers in the serum of animals surviving infection; see key in *E. G–I,* Levels of interferon (IFN) α, interleukin 15 (IL-15), and IFN-γ measured in the serum of all infected animals; mean group values are shown with SDs. Day 9 represents time of euthanasia samples in the nonsurvivor group. **P* < .01; †*P* < .0001 (2-way analysis of variance with Sidak posttest analysis for comparison between survivors and nonsurvivors). Abbreviations: NHP, nonhuman primate; TCID50, median tissue culture infective dose; VSV-EBOV, VSV-based EBOV vaccine; VSV-MARV, VSV-based Marburg virus vaccine.

Unexpectedly, the group that was treated twice with VSV-MARV had a 67% survival rate (Figure 1*C*). The earlier study included only a single VSV-MARV–immunized animal that succumbed to EBOV infection on day 8 [11]. There are significant differences between these studies, such as the challenge virus, the number of treatments (1 vs 2 injections), and the actual VSV-MARV construct (VSV-MARV expressing the Musoke vs the Angola GP). Both GPs vary minimally in amino acid sequence yet appear to possess different immunostimulatory properties because they caused different disease outcome in *Stat1*$^{-/-}$ mice [20]. The outcome of both studies raises questions as to the importance of VSV-driven unspecific and/or filovirus antigen-specific immune responses for the success of postexposure treatment of an EBOV infection. Larger animal groups are needed to answer those questions.

As previously shown, prophylactic protection against EBOV infection through VSV-EBOV vaccination is mainly conferred through EBOV GP–specific antibody responses [21]. In the current study, EBOV GP–specific IgM antibody responses were undetectable or very low over the first 9 days after EBOV challenge and did not reveal any difference between nonsurvivors (Figure 1*D*) and survivors (Figure 1*E*). However, as early as day 9 after infection, EBOV GP–specific IgM levels increased in the survivors at about the same time EBOV GP–specific IgG could be detected in the serum of these animals (Figure 1*F*). In the following weeks, EBOV GP–specific IgG titers increased in all surviving animals (Figure 1F), which also developed VSV-specific IgG (data not shown). In addition, NHPs 10 and 11 developed a MARV GP–specific IgG immune response (data not shown). The observed EBOV GP–specific IgM and IgG responses in these macaques correlate well with levels for both

Table 1. Clinical Findings

Animal	Day 1–5	Day 6	Day 7–9	Day 10–14	Day 21	Time of Death
NHP 1 (1 h)	Neutrophilia	Fever, lymphopenia, Alb↓, AST↑	Moderate rash, thrombocytopenia, Alb↓↓↓, ALT↑↑↑, AST↑↑↑, GGT↑, SUN↑↑, CRE↑↑	. . .	. . .	Day 9
NHP 2 (1 h)	. . .	Alb↓, AST↑	Mild rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑, GGT↑↑↑, SUN↑↑, CRE↑	Mild rash, Alb↓↓↓, AST↑, GGT↑	Alb↓↓	Survived
NHP 3 (1 h)	Neutrophilia	Fever, Alb↓, AST↑	Mild rash, thrombocytopenia Alb↓↓, AST↑↑↑, ALT↑↑↑, GGT↑, SUN↑	Mild rash, Alb↓↓, AST↑↑, ALT↑, SUN↑	Mild rash, Alb↓↓↓, AST↑	Day 28
NHP 4 (1 and 24 h)	Neutrophilia	Fever, Alb↓, AST↑	Mild rash, Alb↓, AST↑↑↑, ALT↑, GGT↑↑, SUN↑	Mild rash, Alb↓↓, AST↑, GGT↑↑	Alb↓	Survived
NHP 5 (1 and 24 h)	Neutrophilia	Moderate rash, thrombocytopenia, Alb↓, AST↑↑↑, GGT↑, SUN↑, CRE↑	Mild rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑↑↑, GGT↑↑, SUN↑↑↑, CRE↑↑↑	. . .	. . .	Day 7
NHP 6 (1 and 24 h)	Neutrophilia	Mild rash, lymphopenia, Alb↓, AST↑↑,	Moderate rash, thrombocytopenia, lymphopenia, Alb↓↓, AST↑↑↑, ALT↑, GGT↑, SUN↑↑↑, CRE↑↑↑	. . .	. . .	Day 8
NHP 7 (24 h)		Alb↓, AST↑	Mild rash thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑↑↑, GGT↑↑, SUN↑	Alb↓, ALT↑, GGT↑	. . .	Survived
NHP 8 (24 h)	Lymphopenia, fever	Thrombocytopenia, Alb↓, AST↑,	Mild rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑↑↑, GGT↑, SUN↑, CRE↑	Mild rash, Alb↓↓, AST↑	Alb↓↓	Survived
NHP 9 (24 h)	Neutrophilia	Alb↓↓, AST↓↓	Moderate rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑↑↑, SUN↑	. . .	. . .	Day 9
NHP 10 (VSV-MARV)	Thrombocytopenia	Alb↓	Alb↓↓, AST↑	Alb↓↓	. . .	Survived
NHP 11 (VSV-MARV)	Thrombocytopenia	Alb↓, AST↑	Alb↓, AST↑↑↑, ALT↑, SUN↑	Alb↓↓, AST↑	Alb↓	Survived
NHP 12 (VSV-MARV)	. . .	Alb↓↓	Moderate rash, thrombocytopenia, Alb↑↑, AST↑↑↑, ALT↑↑↑, GGT↑, SUN↑↑↑, CRE↑↑↑	. . .	. . .	Day 9
Control 1	Neutrophilia, fever	AST↑↑↑, ALT↑, SUN↑, CRE↑	Mild rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑, GGT↑, SUN↑↑↑, CRE↑	. . .	. . .	Day 9
Control 2	Neutrophilia	Alb↓, AST↑	Thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑↑↑, GGT↑, SUN↑↑↑, CRE↑	. . .	. . .	Day 9
Control 3	Neutrophilia	Fever, mild rash, Alb↓, AST↑↑	Moderate rash, thrombocytopenia, Alb↓↓, AST↑↑↑, ALT↑, GGT↑, SUN↑	Moderate rash, thrombocytopenia, Alb↓↓↓, AST↑↑↑, ALT↑↑, GGT↑↑, SUN↑↑↑, CRE↑	. . .	Day 10

Abbreviations: Alb, albumin; ALT, alanine aminortransferase; AST, aspartate aminotransferase; CRE, creatinine; GGT, γ-glutamyltransferase; NHP, nonhuman primate; SUN, serum urea nitrogen; VSV-MARV, vesicular stomatitis virus–based Marburg virus vaccine.

[a] For Alb, ↓, ↓↓, and ↓↓↓ represent mild, moderate, and severe hypoalbuminemia, respectively. For ALT, AST, CRE, GGT, and SUN, ↓, ↓↓, and ↓↓↓ represent 2–3-fold, 4–5-fold, and >5-fold decreases, respectively, and ↑, ↑, and ↑ represent 2–3-fold, 4–5-fold, and >5-fold increases.

classes of antibody detected in a human treated with VSV-EBOV after work exposure in Sierra Leone [19].

Although EBOV-specific antibodies were detected in all survivors, initial protection is unlikely to be due to specific antibodies from vaccination, because the VSV-MARV postexposure treatment resulted in similar protection from EBOV challenge (Figure 1*C*). This finding suggests that robust nonspecific innate immune responses are critical for protection early during EBOV infection. In a previous report of study focusing on the fast-acting potential of VSV-EBOV as a prophylactic vaccine, Marzi et al [6] proposed that innate immune responses from macrophages and dendritic cells and possibly natural killer (NK) cell activation induced by vaccination may play an important role in protection.

Therefore, we analyzed cytokine levels suggestive of NK cell activation, such as IFN-α, interleukin 15 (IL-15), and IFN-γ, in the serum of all animals during the first 14 days following EBOV infection and VSV-EBOV treatment. For this, animals were divided into nonsurvivors and survivors (survivors include NHP 3, which had to be euthanized on day 28) and were compared with the untreated control animals. Up to day 3 after EBOV infection, IFN-α was at low levels (up to 100 pg/mL) in all animals; subsequently, it increased to higher levels with no statistical significant difference between the 3 groups (Figure 1*G*). After postinfection day 6, the amount of IFN-α decreased in the survivors, probably as a result of controlling the EBOV infection, but it significantly increased in the nonsurvivors and the untreated controls, with no difference between those groups (Figure 1*G*).

The low levels of IFN-α early after VSV treatment are unexpected, because VSV is known to be a strong inducer of innate immune responses [22, 23]. However, EBOV encodes 2 proteins (VP24 and VP35) known to strongly antagonize the host type I IFN response to infection [24, 25], probably explaining the observed pattern. Macrophages, early target cells of EBOV infection [26] as well as VSV-EBOV infection, are involved in the production of IFN-α and IL-15, which are known to activate NK cells. resulting in IFN-γ production. Similar to levels of IFN-α, IL-15 levels were low early during infection but increased in all animals by day 6 (Figure 1*H*). On day 9, decreased levels of IL-15 were measured in the serum of surviving animals, in contrast to nonsurvivors (Figure 1*H*).

IFN-γ production was low in all animals until day 6 after EBOV infection and remained low in the surviving animals (Figure 1*I*). In contrast, the nonsurvivors and untreated controls showed significantly increased IFN-γ serum levels by the time of euthanasia, suggestive of a dysregulated stimulation of IFN-γ–producing cells such as NK cells or certain T cells [27]. The high levels of all 3 cytokines on day 9 in the nonsurvivors and untreated controls are probably associated with the cytokine storm, a hallmark of end-stage Ebola hemorrhagic fever in macaques and humans [14, 28, 29].

In conclusion, VSV-EBOV remains highly efficacious as a fast-acting prophylactic vaccine with promising potential for ring/emergency vaccination around the time of exposure. This study added further support for a role of VSV-based filovirus vaccines (VSV-EBOV and VSV-MARV) in treatment if administered early after EBOV or MARV exposure. Protection seems to be initially triggered by a VSV-driven antigen-unspecific innate immune response, including macrophage activation and NK cell-mediated control of EBOV infection that subsequently might be strengthened by an adaptive antigen-specific response. The postexposure treatment window seems short, however, placing this approach behind other promising treatment options [30], such as monoclonal antibodies [31], small interferingRNA [32], and certain small molecule inhibitors [33]. Postexposure treatment with VSV-based filovirus vaccines might be more beneficial in combination with classic antiviral treatment approaches that interfere with EBOV replication, but not with monoclonal antibody therapy, which is GP specific and will neutralize the VSV vector. More research is needed to further evaluate the therapeutic potential of VSV-EBOV.

Notes

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