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Research paper

Single low-dose VSV-EBOV vaccination protects cynomolgus macaques from lethal Ebola challenge

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ABSTRACT

Background: Ebola virus (EBOV), variant Makona, was the causative agent of the 2013–2016 West African epidemic responsible for almost 30,000 human infections and over 11,000 fatalities. During the epidemic, the development of several experimental vaccines was accelerated through human clinical trials. One of them, the vesicular stomatitis virus (VSV)-based vaccine VSV-EBOV, showed promising efficacy in a phase 3 clinical trial in Guinea and is currently used in the ongoing EBOV outbreak in the northeastern part of the Democratic Republic of the Congo (DRC). This vaccine expresses the EBOV-Kikwit glycoprotein from the 1995 outbreak as the immunogen.

Methods: Here we generated a VSV-based vaccine expressing the contemporary EBOV-Makona glycoprotein. We characterized the vaccine in tissue culture and analyzed vaccine efficacy in the cynomolgus macaque model. Subsequently, we determined the dose-dependent protective efficacy in nonhuman primates against lethal EBOV challenge.

Findings: We observed complete protection from disease with VSV-EBOV doses ranging from 1×10^7 to 1×10^1 plaque-forming units. Some protected animals receiving lower vaccine doses developed temporary low-level EBOV viremia. Control animals developed classical EBOV disease and reached euthanasia criteria within a week after challenge. This study demonstrates that very low doses of VSV-EBOV uniformly protect macaques against lethal EBOV challenge.

Interpretation: Our study provides missing pre-clinical data supporting the use of reduced VSV-EBOV vaccine doses without decreasing protective efficacy and at the same time increase vaccine safety and availability - two critical concerns in public health response.

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Research in context

Evidence before this study

Based on promising vaccine efficacy data in human clinical trials, VSV-EBOV is currently deployed in a ring vaccination approach in response to the ongoing Ebola virus (EBOV) outbreak in the Democratic Republic of the Congo. Previous clinical trials established a single intramuscular dose of 2×10^7 plaque forming units (PFU) for humans; however, only limited data is available supporting the need for such a high vaccine dose. In humans, lower vaccine doses have resulted in reduced EBOV antigen-specific total IgG and neutralizing antibody responses despite overall similar seroconversion rates. Data on protective efficacy following lower-dose immunizations in humans is not available and a correlate for protection has not yet been defined for VSV-EBOV. Cynomolgus macaques are considered the 'gold standard' pre-clinical disease

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model for EBOV countermeasure testing. Here, a VSV-EBOV vaccine dose finding study was initiated using this model to produce pre-clinical efficacy data on low-dose immunization to support public health decision-making.

Added value of this study

The dose finding study revealed that a dose as low as 1×10^1 PFU of VSV-EBOV was still effective in completely protecting cynomolgus macaques from disease following lethal EBOV challenge. Humoral immune responses were not visibly dose-dependent, resulting in high-titered total IgG and neutralizing antibodies directed towards the EBOV glycoprotein.

Implications of all the available evidence

Lower VSV-EBOV vaccine doses would greatly support the implementation of additional prophylactic EBOV vaccination strategies using pop-up and targeted geographic approaches as recently recommended by WHO's Strategic Advisory Group of Experts (SAGE). This would increase vaccine availability and reduce adverse effects of VSV-EBOV. Lower doses may also be considered for the emergency ring vaccination approach in case of a VSV-EBOV vaccine shortage.

1. Introduction

Ebola virus (EBOV) is the causative agent of Ebola virus disease (EVD) with up to 90% case fatality rate in humans and uniform lethality in certain nonhuman primate species such as cynomolgus macaques [1]. EBOV got worldwide attention during the West African outbreak from 2013 to 2016 with almost 30,000 cases and 11,000 deaths [2]. The causative agent of that outbreak was designated EBOV-Makona variant and is nowadays an important EBOV challenge variant used for efficacy testing of therapeutics and vaccines. The EBOV-Makona genome sequence differs from EBOV variants isolated from previous Central African outbreaks by only 3% [3]. Therefore, it was not surprising that therapeutics and vaccines previously developed based on EBOV-Mayinga and EBOV-Kikwit (causative variants of the 1976 and 1995 EBOV outbreaks in the Democratic Republic of the Congo, DRC, respectively) showed efficacy against EBOV-Makona in pre-clinical studies [4,5]. Several of these experimental vaccine and treatment approaches were accelerated into human clinical trials in West Africa [4–7].

Among the most promising vaccine candidates was a recombinant vesicular stomatitis virus (VSV)-based vector expressing the glycoprotein (GP) of the EBOV-Kikwit variant (VSV-EBOV or rVSV-ZEBOV; here referred to as VSV-$EBOV_{Kik}$). VSV-EBOV has been shown to uniformly protect macaques from lethal disease after a single dose even when vaccinated seven days prior to challenge [8,9]. Based on promising pre-clinical efficacy data, VSV-$EBOV_{Kik}$ was assessed in 2015 in phase 3 clinical trials in Guinea as a ring vaccination approach resulting in promising efficacy [10,11]. More recently, VSV-$EBOV_{Kik}$ had been included in the public health response to the early 2018 EBOV outbreak along the western border of the DRC; currently, it is being used during the lingering outbreak in the northeastern region of the DRC [12].

Previously, we had determined that GP-specific IgG antibody responses to VSV-EBOV (in this case expressing the EBOV-Mayinga GP; VSV-$EBOV_{May}$) vaccination were critical for protection against lethal EBOV challenge in macaques [13]. A similar role for GP-specific antibodies was reported from the VSV-$EBOV_{Kik}$ clinical trials making this response a potential correlate of protection in humans [14,15]. Clinical trials also indicated a vaccine dose-dependent antibody response which ultimately lead to a recommended dose of 2×10^7 plaque forming units (PFU) VSV-$EBOV_{Kik}$ for human use [15–19]. The vaccine dose for ring vaccination in DRC, which had initially been higher, has now also been adjusted to that dose [20]. Unfortunately, the severity of adverse effects to VSV-$EBOV_{Kik}$ immunization may be positively correlated with the vaccine dose even though not all clinical trials support this association [14,21,22].

In this study, we first generated a VSV vector expressing the GP from the more recent EBOV-Makona variant (VSV-$EBOV_{Mak}$) and tested its efficacy in the cynomolgus macaque model. Subsequently, we determined the minimum single vaccine dose required to uniformly protect cynomolgus macaques from lethal homologous EBOV challenge. Surprisingly, we found that a single dose of only 1×10^1 PFU of VSV-$EBOV_{Mak}$ completely protected macaques from disease, whereas doses below 10^1 PFU resulted in vaccine breakthrough with animals succumbing to EBOV-Makona challenge. Interestingly, the GP-specific antibody responses elucidated through vaccination were not visibly dose-dependent. Overall, our data suggests that a lower dose of VSV-EBOV seems as efficacious and may ease adverse effects in vaccinated individuals and alleviate concerns on future vaccine availability.

2. Methods

2.1. Ethics statement

All infectious work with EBOV was performed in the maximum containment laboratory at the Rocky Mountain Laboratories (RML), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. RML is an institution accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All procedures followed standard operating procedures (SOPs) approved by the RML Institutional Biosafety Committee (IBC). Animal 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 Animal Welfare Act, United States Department of Agriculture. The study was approved by the RML Animal Care and Use Committee (ACUC). Procedures were conducted in animals anesthetized by trained personnel under the supervision of veterinary staff. The humane endpoint criteria for euthanasia were specified and approved by the RML ACUC. All efforts were made to ameliorate animal welfare and minimize animal suffering in accordance with the Weatherall report on the use of non-human primates (NHPs) in research (https://royalsociety.org/policy/publications/2006/weatherall-report/). Animals were housed in adjoining individual primate cages that enabled social interactions, under controlled conditions of humidity, temperature, and light (12 h light - dark cycles). Food and water were available *ad libitum*. Animals were monitored and fed commercial monkey chow, treats, and fruit at least twice a day by trained personnel. Environmental enrichment consisted of commercial toys, music, video and social interaction.

2.2. Vaccine vectors

VSV-$EBOV_{Mak}$ was generated by cloning the full-length GP coding region of the EBOV-Makona (GenBank accession number KP096421; bp 6039-8068; 8A version) into the VSVΔG genomic plasmid [23]. Virus recovery, propagation and titration has been previously described [24]. VSV wildtype (VSV_{wt}), VSV-$EBOV_{Kik}$, and VSV expressing the GP of the Marburg virus (MARV), Angola strain (VSV-$MARV_{Ang}$) were previously described [24,25]. Cynomolgus macaques were vaccinated with the indicated doses of VSV-$EBOV_{Mak}$ or VSV-$MARV_{Ang}$ achieved by 10-fold serial dilutions of the vaccine seed stocks (1×10^9 plaque-forming units (PFU)/ml) in

DMEM without supplements. Plaque assay was performed for vaccine back-titration for the low dose groups as previously described [24].

2.3. Challenge virus

EBOV-Makona, Guinea C07 strain (EBOV-Makona) was propagated on VeroE6 cells (mycoplasma negative), titered via plaque-forming unit (PFU) and 50% tissue culture infectious dose ($TCID_{50}$) assays on VeroE6 cells and stored in liquid nitrogen (EBOV-Makona, passage 2) [26]. The infectious material was originally obtained from the National Microbiology Laboratory, Public Health Agency of Canada (Gary Kobinger, Ph.D.).

2.4. Growth kinetics

VeroE6 cells (mycoplasma negative) were grown to confluency in a 12-well plate and infected with a multiplicity of infection (MOI) of 0.01 with VSV_{wt}, VSV-$EBOV_{Kik}$, and VSV-$EBOV_{Mak}$ in triplicates. After one hour, the inoculum was removed and replaced with DMEM supplemented with 2% FBS, penicillin/streptomycin and L-glutamine. Supernatant samples were collected at 0, 6, 12, 24, 36, 48, and 72, hours post-infection and stored at −80 °C until titration. Virus titers from these samples were determined by inoculating VeroE6 cells (mycoplasma negative) with 10-fold serial dilutions in triplicate. 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 and the 50% tissue culture infectious dose ($TCID_{50}$) was calculated for each sample employing the Reed and Muench method [27].

2.5. Macaque study design

A total of 34 cynomolgus macaques (*Macaca fascularis*), 17 male and 17 female animals, 3–6 years of age and 3–8.5 kg in weight, were used in this study. The macaques were randomly divided into 7 study groups of 4 (2 females and 2 males) and 6 control animals (3 females and 3 males). The study animals were immunized intramuscularly (i.m.) with 1×10^{-1} to 1×10^{7} PFU VSV-$EBOV_{Mak}$ into 2 sites in the caudal thighs; the low-dose inocula were confirmed by back-titration using a PFU-based infectivity assay (Table S1). The control animals received 1×10^{7} PFU VSV-$MARV_{Ang}$ via the same route. The study was split into 5 segments due to space and manpower restrictions in the BSL4 laboratory. The segmentation of the study also allowed to minimize the use of NHPs and was performed as follows: (1) $n = 2$ controls and $n = 4$ 1×10^{7} PFU; (2) $n = 2$ controls, $n = 4$ 1×10^{6} PFU, $n = 4$ 1×10^{4} PFU and $n = 4$ 1×10^{2} PFU; (3) $n = 1$ control and $n = 4$ 1×10^{1} PFU; (4) $n = 1$ control and $n = 4$ 1×10^{0} PFU; and (5) $n = 4$ 1×10^{-1} PFU. All animals were challenged i.m. on day 0 with a lethal dose of 1×10^{4} $TCID_{50}$ (1000 LD_{50}) EBOV-Makona into 2 sites in the caudal thighs as described previously [9]. The challenge virus titer was confirmed by back-titration on VeroE6 cells performing $TCID_{50}$ determination as described above. For all study segments the back-titration resulted in 1.8×10^{4} $TCID_{50}$/ml. Physical examinations and blood draws were performed on days −28, −14, 0 (challenge day), 3, 6, 9, 14, 21, 28, and 35 and at euthanasia (day 42 – survivors; humane endpoint for non-survivors). The animals were observed at least twice daily for clinical signs of disease using RML ACUC-approved criteria. Following euthanasia, a necropsy was performed and samples of key tissues (liver, spleen and lymph nodes) were collected for virologic and pathological analysis.

2.6. Hematology and serum chemistries

The platelet count was determined from EDTA blood with the IDEXX ProCyte DX analyzer (IDEXX Laboratories, Westbrook, ME). Serum biochemistry (including AST) was analyzed using the Piccolo Xpress Chemistry Analyzer and Piccolo General Chemistry 13 Panel discs (Abaxis, Union City, CA).

2.7. Viremia

Virus loads in macaque blood samples were determined using a $TCID_{50}$ assay on VeroE6 cells (mycoplasma negative) as described above under “2.4 Growth kinetics”.

2.8. Assessment of humoral immune response

Post-challenge NHP sera were inactivated by gamma-irradiation (5 MRad) and removed from the maximum containment laboratory according to RML SOP approved by the RML IBC. This inactivation method is well-established and has been shown to minimally impact serum antibody binding [28,29]. Antibody titers were determined using enzyme-linked immunosorbent assay (ELISA) kits based on recombinant soluble EBOV-Makona GPΔTM (Alpha Diagnostics, San Antonio, TX). For this, serum samples were diluted 1:100 (IgM), 1:500 (pre-challenge IgG) or 1:1000 (post-challenge IgG). The ELISA was performed, and titers were calculated as per manufacturer's instructions. Neutralizing antibody titers were determined by 50% focus reduction neutralization test ($FRNT_{50}$) using EBOV-Mayinga expressing green fluorescence protein (EBOV-Mayinga-GFP) as previously described [9].

2.9. Serum cytokine levels

Post-challenge macaque sera were treated by gamma-irradiation (5 MRad) and removed from the maximum containment laboratory according to a SOP approved by the RML IBC. 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 analytes (IFNγ, IL-6, IL-1ra, IL-2, IL-10, IL-15, MCP-1, TGFα, sCD40L and TNFα) were determined for all samples using the Bio-Plex 200 system (BioRad Laboratories Inc.).

2.10. Histopathology and immunohistochemistry

Histopathology and immunohistochemistry were performed on small macaque tissue samples. After fixation/inactivation for 7 days in 10% neutral buffered formalin, small tissue samples were removed from high containment according to a SOP approved by the RML IBC. Subsequently, tissue samples were embedded in paraffin and tissue sections were stained with hematoxylin and eosin (H&E). To detect EBOV antigen, immunohistochemistry was performed using an anti-VP40 polyclonal rabbit serum (kindly provided by Yoshihiro Kawaoka, University of Wisconsin-Madison) as previously described [26].

2.11. Statistical analysis

Statistical analysis was performed in Prism 7 (GraphPad). Data presented in Fig. S1 were examined using two-way ANOVA with Tukey's multiple comparison to evaluate statistical significance at all timepoints between all groups. Significant differences in the survival curves shown in Fig. 1A were determined performing Log-Rank analysis. Statistical significance is indicated as follows: $p < 0.0001$ (****), $p < 0.001$ (***), $p < 0.01$ (**) and $p < 0.05$ (*).

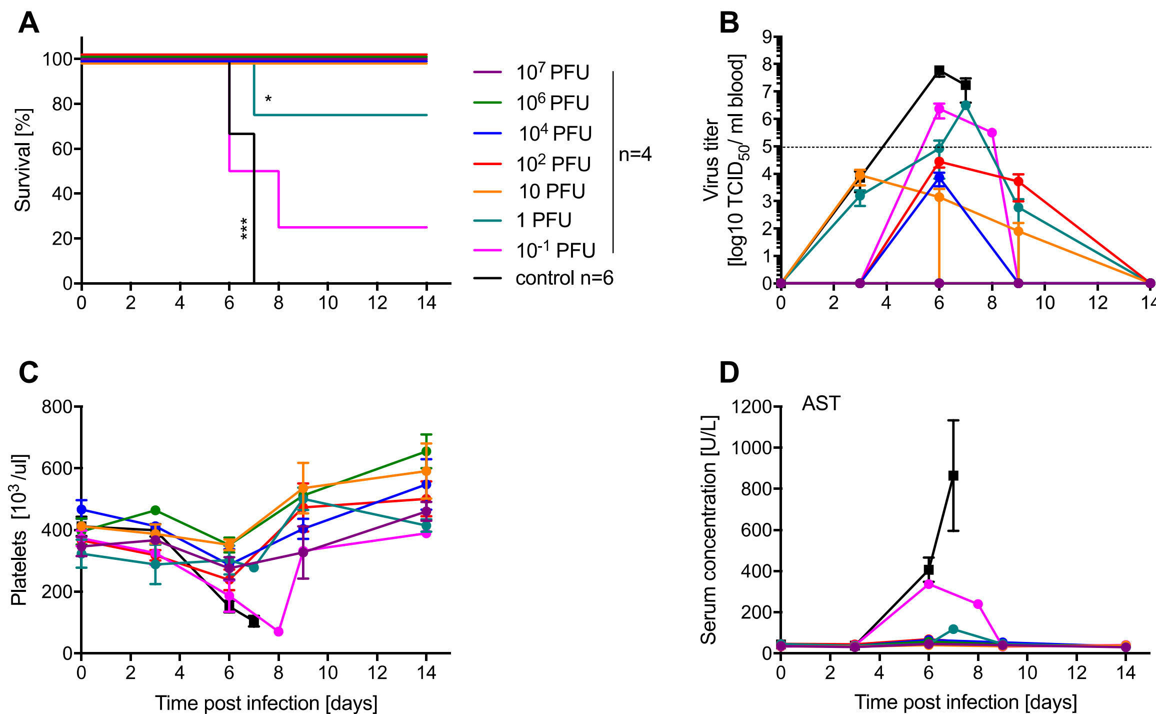

Fig. 1. Survival and clinical parameters after EBOV infection. (A) Survival curves and (B) virus titers are presented for each vaccine dose group. Dotted line indicates threshold for viremia associated with fatal disease. (C) Whole blood platelet count and (D) aspartate aminotransferase (AST) levels in serum samples collected at every exam day and the time of euthanasia are shown for each vaccine group. Error bars indicate standard error of the mean. Statistical significance is indicated (* $p < 0.05$; *** $p < 0.001$).

3. Results

3.1. Generation and characterization of VSV-EBOV$_{Mak}$

VSV-EBOV$_{Mak}$ was constructed by replacing the open reading frame of the VSV glycoprotein G by that of the EBOV-Makona GP. Recombinant viruses were recovered using the VSV reverse genetics system and subsequently propagated and titered in VeroE6 cells as previously described [24,30,31]. VSV-EBOV$_{Mak}$ growth kinetics were compared to those of VSV$_{wt}$ and VSV-EBOV$_{Kik}$ on VeroE6 cells. VSV$_{wt}$ displayed a faster growth kinetic than both EBOV GP-expressing VSV vectors with a statistically significant difference at 24 h (Fig. S1). VSV-EBOV$_{Mak}$ showed a slightly reduced growth kinetic compared to VSV-EBOV$_{Kik}$ (Fig. S1); however, the difference was not statistically significant.

3.2. Protective efficacy of VSV-EBOV$_{Mak}$ in cynomolgus macaques

Initially, VSV-EBOV$_{Mak}$ was used to i.m. vaccinate a group of cynomolgus macaques ($n = 4$) with the standard dose of 1×10^7 PFU (Table S1). Control animals ($n = 2$) received 1×10^7 PFU of VSV-MARV$_{Ang}$ [25,32] by the same route (Table S1). Four weeks after vaccination all macaques were i.m. challenged with 1×10^3 PFU of EBOV-Makona, a standard challenge dose [26]. Animals were scored at least twice daily for clinical signs of disease and euthanized at day 42 post challenge (protected animals) or when a predetermined humane endpoint was reached (non-protected animals). Frequent physical examinations were performed including a blood draw to perform virology, serology, hematology and blood chemistry analyses. All VSV-EBOV$_{Mak}$ vaccinated animals were completely protected from disease, never developed EBOV-Makona viremia, nor did they show any hallmark blood parameters of EBOV infection such as elevation of aspartate aminotransferase (AST) or thrombocytopenia (Fig. 1; Fig. S2; Table S1). In contrast, all control animals had to be euthanized on days 6 and 7 with severe clinical signs resembling EVD, high EBOV-Makona viremia as determined by TCID$_{50}$ assay, severe thrombocytopenia and elevated AST levels (Fig. 1; Fig. S2; Table S1).

3.3. VSV-EBOV$_{Mak}$ dose finding in cynomolgus macaques

Having established the pre-clinical efficacy of VSV-EBOV$_{Mak}$ in the cynomolgus macaque model, we next performed a vaccine dose finding study with the aim to define the minimum dose that still provides full protection from disease against lethal homologous EBOV-Makona challenge. Six groups of cynomolgus macaques ($n = 4$) were vaccinated i.m. with doses of VSV-EBOV$_{Mak}$ ranging from 1×10^6 to 1×10^{-1} PFU (Table S1). A control group ($n = 4$) received 1×10^7 PFU of VSV-MARV$_{Ang}$ by the same route (Table S1). EBOV-Makona challenge (1×10^3 PFU) by the i.m. route was performed 28 days after vaccination and animals were followed as described above. Surprisingly, we found uniform protection with no clinical signs of disease in animals that were vaccinated with a dose as low as 1×10^1 PFU VSV-EBOV$_{Mak}$ (Fig. 1; Fig S2; Table S1). EBOV-Makona viremia was not detected in animals vaccinated with 1×10^6 PFU of VSV-EBOV$_{Mak}$, in all other protected dose groups (1×10^4, 1×10^2 and 1×10^1 PFU), most animals presented with transient, low-level viremia ($<10^5$ TCID$_{50}$/ml) that was cleared by days 9 or 14 post challenge (Fig. 1B; Fig. S2). We did not observe hallmark features of EVD, such as thrombocytopenia or elevated AST levels, with any protected animal (Fig. 1C and D). Vaccine breakthrough was observed with doses of 1×10^0 and 1×10^{-1} PFU VSV-EBOV$_{Mak}$ resulting in 75 and 25% survival, respectively (Fig. 1; Fig. S2; Table S1). All animals succumbing to EBOV-Makona challenge presented with typical clinical signs resembling EVD including high viremia, severe thrombocytopenia and elevated AST levels (Fig. 1; Fig. S2; Table S1).

3.4. Humoral immune responses

EBOV-Makona GP-specific IgM and IgG responses were measured by ELISA using a commercial kit following the

manufacturer's instructions. Surprisingly, we did not observe a discernable dose-dependent effect on EBOV GP-specific IgM or IgG titers after vaccination (day −14 and day 0) in surviving animals (Fig. 2A–C; Fig. S3). However, within all groups receiving 1×10^4 PFU or less of VSV-EBOV$_{Mak}$, we observed a less uniform antibody response among individual animals compared to animals of groups vaccinated with 1×10^6 or 1×10^7 PFU (Fig. 2A-C and; Fig. S3). Neutralizing responses were measured by FRNT$_{50}$ using EBOV-Mayinga-GFP. At the time of challenge (day 0), neutralizing responses were either not detected or very low in FRNT$_{50}$ titers. At day 42, all protected animals showed detectable neutralizing responses, but like the IgG responses there was no significant difference in animals belonging to the different groups (Fig. 2D). Noteworthy, only animals lacking GP-specific IgM and IgG or and neutralizing responses went on to develop severe disease and had to be euthanized; these were all animals in the control group as well as one and three animals in the 1×10^0 and 1×10^{-1} PFU vaccine groups, respectively (Figs. 1 and 2; Fig. S3). Interestingly, even the one protected animal in the 1×10^{-1} PFU group developed similar strong anti-EBOV GP responses, as did protected animals in the groups vaccinated with higher VSV-EBOV$_{Mak}$ doses (Figs. 1 and 2; Fig. S3). For all the protected animals the challenge with EBOV-Makona acted as a boost vaccination resulting in a substantial increase in EBOV GP-specific IgG between days 6 and 9 post challenge (Fig. 2C; Fig. S3), a response that had been observed previously [9,13].

3.5. Other immune responses

Analysis of T cell responses was not performed due to lack of data supporting a critical role of cell-mediated immunity in VSV-EBOV vaccination [13,33]. Instead we measured innate immune responses by determining cytokine levels prior to vaccination (day −28), prior to challenge (day 0) and shortly after challenge (day 3). All study animals had baseline responses prior to vaccination indicative of a non-activated status (Fig. 3). Prior to challenge, we either could not detect responses (i.e. interferon gamma (IFNγ), interleukin 6 (IL-6) and tissue necrosis factor alpha (TNFα)) or detected activated responses with no distinctions among animals (i.e. IL-1 receptor antagonist (IL-1ra), IL-2, IL-10, IL-15, monocyte chemoattractant protein-1 (MCP-1), transforming growth factor alpha (TGFα) and soluble CD40 ligand (sCD40L)), indicating a certain degree of activation but no difference among vaccinated and control groups (Fig. 3). Three days post EBOV-Makona challenge, responses were elevated without a difference to those at day 0 for most of the presented cytokines except for IFNγ, IL-6 and to some degree TNFα (Fig. 3). Interestingly, all macaques vaccinated with the two highest doses of VSV-EBOV$_{Mak}$ (1×10^7 and 1×10^6 PFU) that lacked evidence for challenge virus replication (Fig. 1B, Fig. S2) showed no IFNγ and TNFα responses post challenge (Fig. 3). Other analyzed mediators did not show an increase in expression at any of the three tested time points (day −28, day 0 and day 3).

3.6. Pathologic changes in protected and non-protected cynomolgus macaques

All non-protected animals developing severe disease were euthanized according to established humane endpoint criteria. Characteristic gross pathology changes for EBOV infection in macaques were noticed as previously described [26,34,35]. Tissue from key target organs (liver, spleen and inguinal lymph node) were collected at the time of euthanasia. These organs revealed high EBOV-Makona titers determined by TCID$_{50}$ infectivity assay on Vero E6 cells with no significant difference among non-protected animals (Fig. S4). As previously published [26,34,35], histopathology revealed EBOV-typical lesions showing multifocal to coalescing hepatic necrosis with acute inflammation and abundant fibrin microthrombi within the hepatic sinusoids as well as EBOV antigen expression (Figs. S5A, B). Similarly, necrosis (lymphocytolysis) of the white pulp, abundant fibrin within the red pulp, multifocal acute inflammation and EBOV antigen expression were observed in the spleen (Figs. S6A, B). All protected animals were euthanized at day 42, the study end point, except for the survivor in the 1×10^{-1} PFU group, which was euthanized on day 14 post challenge as a sole survivor due to animal welfare reasons. In contrast to the non-protected animals, pathology and histopathology did not reveal any abnormal changes and EBOV-Makona antigen could not be detected in liver and spleen of any surviving animal (Figs. S5C–F; Fig. S6C–F).

4. Discussion

In this study we generated a more contemporary VSV-based EBOV vaccine by expressing the GP of EBOV-Makona, the causative strain of the West African epidemic [3]. *In vitro* studies revealed similar characteristics of VSV-EBOV$_{Mak}$ and VSV-EBOV$_{Kik}$ (Fig. S1). When macaques were vaccinated with 1×10^7 PFU, the standard vaccination dose for VSV-EBOV in pre-clinical NHP studies [8,13], we observed sterile protection from clinical disease (Fig. 1A; Fig. S2; Table S1). The EBOV GP-specific antibody responses in macaques were similar to what has been published before from NHPs vaccinated with VSV-EBOV$_{Kik}$ and VSV-EBOV$_{May}$ (Fig. 2) [9,13] suggesting that VSV-EBOV$_{Mak}$ is similarly effective as any other previous VSV-EBOV vector.

The initial VSV vectored vaccine work for EBOV was based on VSV-EBOV$_{May}$ expressing the GP derived from the prototype EBOV-Mayinga strain [8,31]. The majority of pre-clinical NHP efficacy studies have used this vaccine vector in homologous (EBOV-Mayinga) and heterologous (EBOV-Kikwit) challenge experiments [36]. The generation of VSV-EBOV$_{Kik}$ reflected an adaptation to the EBOV strain that had caused the biggest outbreak recorded at the time (Kikwit, DRC, 1995) [37]. The switch to VSV-EBOV$_{Mak}$ is an adjustment driven by the West African outbreak. Whatever adjustment will be done, it is unlikely to provide a homologous vaccine to any future EBOV strain causing an outbreak (see 2018/2019 outbreaks in DRC; EBOV-Ituri). A comparison of known EBOV GP sequences has shown an amino acid variation ranging from 2 to 3.5% (Table S2). Almost all mutations are located within the mucin-like domain [38], which is dispensable for virus entry and the induction of protective immune responses [39]. Thus, one can expect that any of these VSV-EBOV vectors will induce cross-protective anti-GP responses against EBOV strains and unlikely to affect vaccine efficacy. This was demonstrated with sera from NHPs vaccinated with VSV-EBOV$_{ik}$that showed indistinguishable neutralizing antibody titers to three EBOV strains [9]. Furthermore, countermeasures effective against EBOV-Makona, such as VSV-EBOV$_{Kik}$ and monoclonal antibodies, are currently deployed against the current DRC EBOV outbreak strain [40,41].

A goal of this study was to determine the minimal vaccine dose required to protect macaques from lethal EBOV challenge. When we vaccinated groups of macaques with VSV-EBOV$_{Mak}$ doses ranging from 1×10^6 to 1×10^{-1} PFU, we were surprised to find that a vaccine dose as little as 1×10^1 PFU resulted in uniform protection from clinical disease (Fig. 1; Table S1). Transient challenge virus viremia (cleared between days 9 and 14 post challenge) was only detected in protected animals of the lower dose vaccine groups. However, titers never reached 10^5 TCID$_{50}$/ml blood, a previously established threshold for protection from disease (Fig. S2, Table S1) [26,42]. Only non-protected animals including the control group macaques developed high challenge virus viremia ($>10^6$ TCID$_{50}$/ml blood; Fig. S2) and severe disease as indicated by hallmark clinical parameters (Fig. 1C and D) and needed to be euthanized. Thus, a

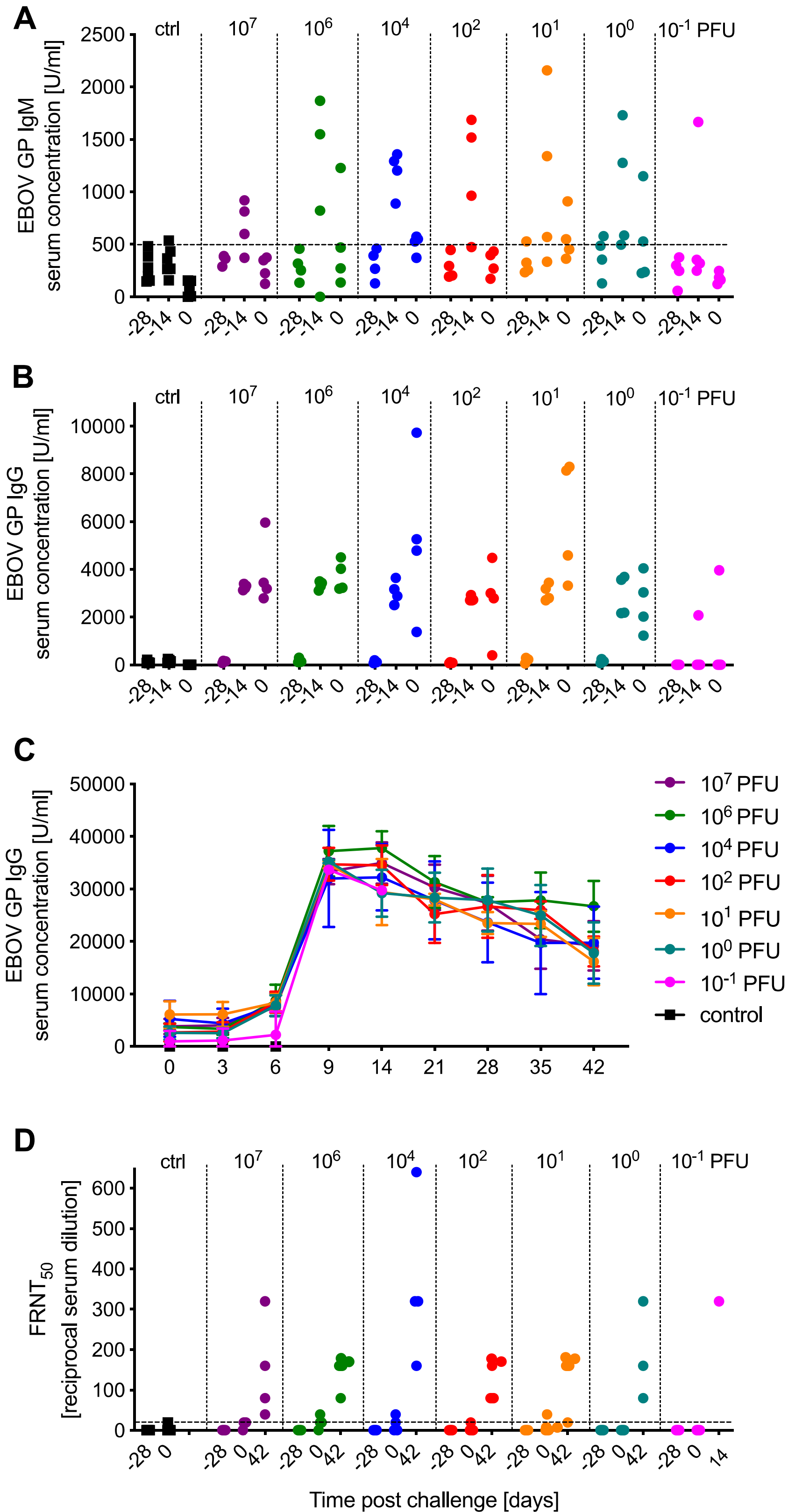

Fig. 2. Serology after vaccination and challenge. (A) EBOV-Makona GP-specific IgM serum concentrations after vaccination. (B) EBOV-Makona GP-specific IgG serum concentrations after vaccination. (C) EBOV-Makona GP-specific IgG serum concetrations after challenge at every exam day and the time of euthanasia. Error bars indicate standard deviation. (D) EBOV neutralizing titers are presented. Serum dilution reducing 50% of the focus number are shown for individual animals at the time of vaccination (day −28), challenge (day 0) and for survivors (day 42). Note, dotted lines indicate the limit of detection.

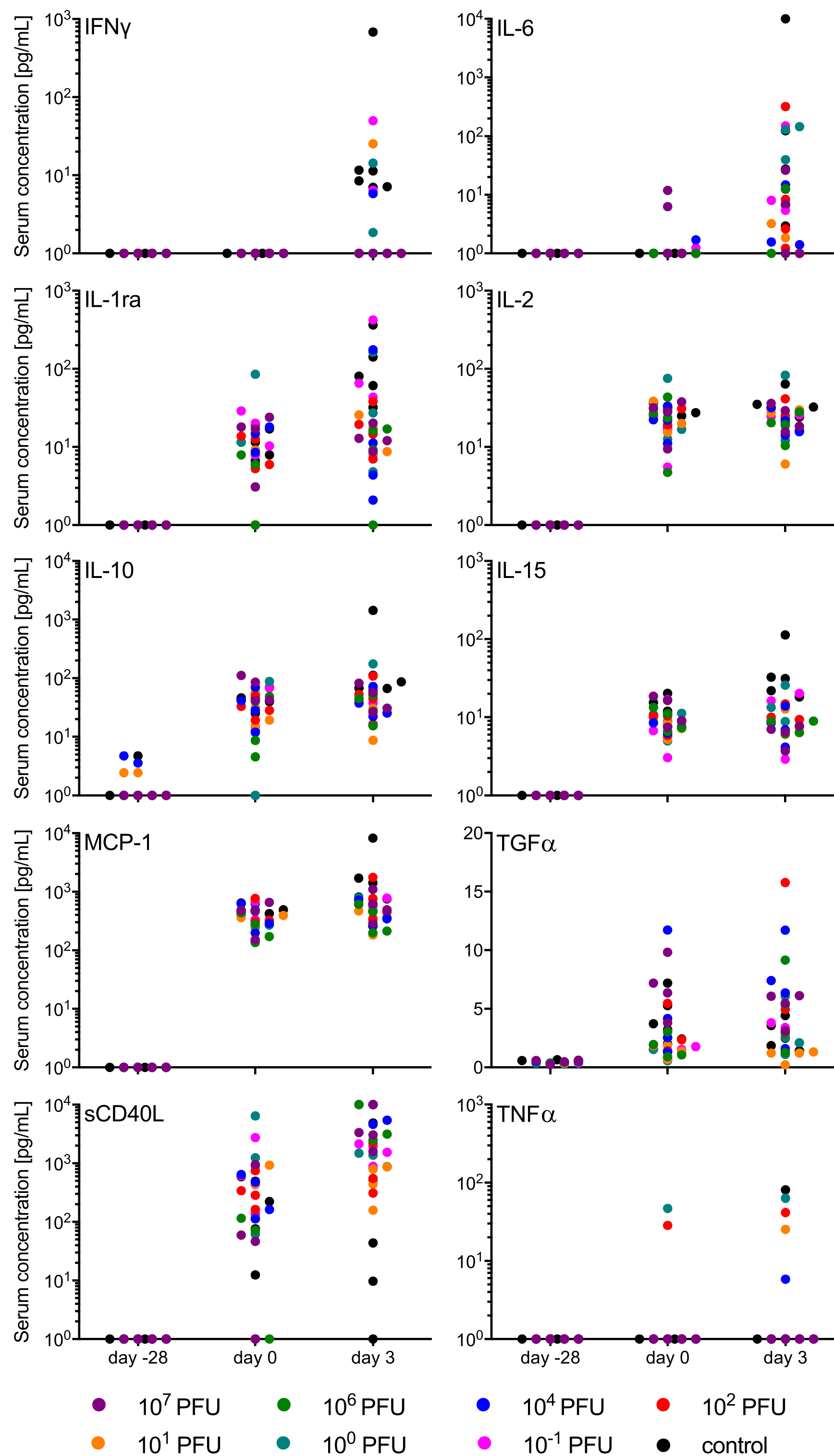

Fig. 3. Cytokine responses in vaccinated and challenged NHPs. Serum concentrations for IFNγ, IL-6, IL-1ra, IL-2, IL-10, IL-15, MCP-1, TGFα, sCD40L and TNFα were determined at the time of vaccination (day −28), challenge (day 0) and early after EBOV-Makona challenge (day 3). Each dot represents an individual animal at the indicated time point.

remarkably low VSV-EBOV dose is sufficient to protect macaques from EBOV challenge similar to what had been published earlier for VSV-EBOV in mice [43] but had never been evaluated in NHPs, the ultimate pre-clinical EBOV animal disease model.

We have previously shown that the humoral immune response to EBOV GP plays a critical role for protection [13]. Furthermore, we established that the total IgG response to EBOV GP is an important parameter and likely a good correlate of protection for pre-clinical studies [9]. The clinical trials using the VSV-EBOV$_{Kik}$ vaccine confirmed the importance of the total IgG response to EBOV GP as a likely correlate of protection, but some studies reported dose-dependent GP-specific IgG responses to VSV-EBOV$_{Kik}$ vaccination [15–19]. Interestingly, in the macaque study here we found no such correlation between vaccine dose and total EBOV GP-specific

IgG or neutralizing immune responses (Fig. 2; Fig. S3). In cynomolgus macaques it appears to be rather an "all-or-nothing" humoral immune response as best indicated for the survivors and non-survivors in the groups vaccinated with the two lowest vaccine doses (Fig. 2; Fig. S3). Further investigations need to confirm a difference in dose-dependent immune responses between humans and macaques and the potential mechanism behind it.

In this study, we did not focus on T cell-mediated immune responses upon vaccination with VSV-$EBOV_{Mak}$ as those have previously been considered of limited importance for macaques and mice vaccinated with VSV-EBOV vectors [8,13,33,43]. In addition, there is currently only limited data on T cell-mediated immune responses in human EBOV vaccinees [44], with one report suggesting a role for circulating follicular T helper cells [45]. To see if protection was associated with activated innate immune responses, we performed cytokine analysis on serum samples collected prior to vaccination, at the day of challenge and shortly after challenge. Overall, the varying vaccine doses did not result in any significant differences regarding production of immune mediators. Notable, only the animals in the group with the two highest vaccine doses did not have IFNγ and TNFα responses upon challenge indicative of non-replicating challenge virus similar to what was published before [8] and in line with viremia data presented here (Fig. 1B; Fig S2). Thus, as expected from the vaccination schedule used in this study (immunization 28 days prior to challenge) we likely can exclude the influence of innate mediators in the outcome of this dose-down vaccine protection study.

Currently, the recommended human dose for VSV-$EBOV_{Kik}$ vaccination is 2×10^7 PFU by single site i.m. administration [15–19]. This dose is given for any vaccination approach including ring vaccination [9–11]. Recently, it was endorsed to consider additional pop-up and targeted geographic vaccination approaches to provide broader vaccine coverage in the general population with no known EBOV exposure [46]. With ongoing ring vaccination as the primary public health vaccine response this could result in vaccine shortage unless production can be scaled up or a second vaccine candidate can be deployed. One way to reduce the burden on vaccine production would be to lower the vaccine dose. This seems conceptionally achievable as VSV-EBOV is a live-attenuated, replication-competent vaccine and thus seem to be less dependent on high vaccine doses [46,47]. The feasibility of such an approach is supported by the preclinical data here with immune responses in the macaques that were not visibly dose-dependent (Fig. 2; Fig. S3). In humans, a vaccine dose of 3×10^5 PFU VSV-EBOV has resulted in reduced GP-specific IgG and neutralizing antibody responses despite overall similar seroconversion rates [22]. However, data on protective efficacy following lower-dose immunization in humans is not available and a correlate for protection in humans has not yet been defined. By determining the recommended vaccine dose for VSV-EBOV, the clinical trials (phase 1–3) seem to have rather erred on the side of caution with a higher vaccine dose, a plausible approach. Based on data presented here, however, lower-dose vaccination could be considered for these additional approaches. If considered, logistic questions on how to disseminate lower vaccine doses need to be resolved. Feasible options might be the administration of lower volumes or vaccine dilution on site as manufacturing lower-dose vaccine vials is a longer lasting and more difficult process. Whether ring vaccination should be performed with lower doses remains open at this time and likely should be addressed in additional preclinical studies or clinical trials first. Should vaccine shortage arise, one could consider lower doses also for this vaccination approach.

VSV-EBOV is a live-attenuated vaccine platform that has in general caused mild adverse effects in humans such as injections site irritation, headache, fatigue, fever, chills, myalgia, and arthralgia [14,21,22]. In particular, the Geneva phase 1 clinical trial has reported more serious reactions to vaccination including arthritis, arthralgia and vesicle formation from which VSV-EBOV could be isolated [22]. In contrast, the larger African phase 2/3 clinical trials have not reported similar severe adverse effects upon vaccination [10,11,48,49] and there seems to be no convincing association with vaccine dose and severity of adverse effects. Adverse effects have never been reported from any animal studies aside from occasional adverse reactions at the injection site. This is not surprising as the relatively mild and often subjective adverse effects described from human vaccinees are difficult to observe and monitor in animals. Nevertheless, lower vaccine doses would rather reduce adverse effects and their severity in vaccinees than the opposite.

Preclinical studies and clinical trials are never the same and therefore it is difficult to make firm recommendations for vaccination of humans. However, the promising efficacy with extremely low vaccine doses and the interesting finding of a non-dose-dependent humoral immune response in macaques indicate that lower vaccine doses may also be protective in humans. Reduction of the vaccine dose would automatically increase the current vaccine stocks, likely increase vaccine safety and ease the burden on future vaccine production.

Declaration of Competing Interest

H.F. claims intellectual property regarding the vesicular stomatitis virus-based filovirus vaccines. All other authors declare no conflict of interests.

CRediT authorship contribution statement

Andrea Marzi: Conceptualization, Project administration, Funding acquisition, Supervision, Writing - original draft, Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Pierce Reynolds:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Reinaldo Mercado-Hernandez:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Julie Callison:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Friederike Feldmann:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Rebecca Rosenke:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Tina Thomas:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Dana P. Scott:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Patrick W. Hanley:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Elaine Haddock:** Methodology, Resources, Data curation, Formal analysis, Writing - review & editing. **Heinz Feldmann:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing - original draft, Methodology, Resources, Data curation, Formal analysis, Writing - review & editing.

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Data availability

Data supporting the findings of this study are available within the manuscript and supplementary information and are also available from the authors upon request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2019.09.055.

References

[1] Baseler L, Chertow DS, Johnson KM, Feldmann H, Morens DM. The pathogenesis of Ebola virus disease. Annu Rev Pathol 2017;12:387–418.
[2] WHO. Ebola situation report, 30 March 2016 2016. http://apps.who.int/ebola/current-situation/ebola-situation-report-30-march-2016 (accessed).
[3] Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola virus disease in Guinea. New Engl J Med 2014;371(15):1418–25.
[4] Cross RW, Mire CE, Feldmann H, Geisbert TW. Post-exposure treatments for Ebola and Marburg virus infections. Nat Rev Drug Discov 2018;17(6):413–34.
[5] Mire CE, Geisbert TW, Feldmann H, Marzi A. Ebola virus vaccines - reality or fiction? Expert Rev Vaccines 2016;15(11):1421–30.
[6] Cardile AP, Warren TK, Martins KA, Reisler RB, Bavari S. Will there be a cure for Ebola? Annu Rev Pharmacol Toxicol 2017;57:329–48.
[7] Marzi A, Mire CE. Current Ebola virus vaccine progress. BioDrugs 2019;33(1):9–14.
[8] Jones SM, Feldmann H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat Med 2005;11(7):786–90.
[9] Marzi A, Robertson SJ, Haddock E, et al. EBOLA VACCINE. VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain. Science 2015;349(6249):739–42.
[10] Henao-Restrepo AM, Longini IM, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet 2015;386(9996):857–66.
[11] Henao-Restrepo AM, Camacho A, Longini IM, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit!). Lancet 2017;389(10068):505–18.
[12] Branswell H. WHO's Tedros: experimental Ebola vaccine in the DRC has saved countless lives. Stat 2019. https://www.statnews.com/2019/01/04/ebola-vaccine-tedros-drc/.
[13] Marzi A, Engelmann F, Feldmann F, et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. Proc Natl Acad Sci USA 2013;110(5):1893–8.
[14] Regules JA, Beigel JH, Paolino KM, et al. A recombinant vesicular stomatitis virus Ebola vaccine - Preliminary Report. New Eng J Med 2017;376(4):330–41.
[15] Huttner A, Agnandji ST, Combescure C, et al. Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational cohort study. Lancet Infect Dis 2018;18(7):738–48.
[16] Huttner A, Dayer JA, Yerly S, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. Lancet Infect Dis 2015;15(10):1156–66.
[17] Agnandji ST, Fernandes JF, Bache EB, et al. Safety and immunogenicity of rVSVDeltaG-ZEBOV-GP Ebola vaccine in adults and children in Lambarene, Gabon: a phase I randomised trial. PLoS Med 2017;14(10):e1002402.
[18] ElSherif MS, Brown C, MacKinnon-Cameron D, et al. Assessing the safety and immunogenicity of recombinant vesicular stomatitis virus Ebola vaccine in healthy adults: a randomized clinical trial. CMAJ 2017;189(24):E819–EE27.
[19] Heppner DG Jr, Kemp TL, Martin BK, et al. Safety and immunogenicity of the rVSVG-ZEBOV-GP Ebola virus vaccine candidate in healthy adults: a phase 1b randomised, multicentre, double-blind, placebo-controlled, dose-response study. Lancet Infect Dis 2017;17(8):854–66.
[20] soch h. WHO adapts Ebola vaccination strategy in the Democratic Republic of the Congo to account for insecurity and community feedback. 2019. https://www.healthysoch.com/health/general/who-adapts-ebola-vaccination/.
[21] Halperin SA, Arribas JR, Rupp R, et al. Six-Month safety data of recombinant vesicular stomatitis virus-zaire Ebola virus envelope glycoprotein vaccine in a phase 3 double-blind, placebo-controlled randomized study in healthy adults. J Infect Dis 2017;215(12):1789–98.
[22] Agnandji ST, Huttner A, Zinser ME, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and Europe - Preliminary Report. New Eng J Med 2016;374(17):1747–60.
[23] Schnell MJ, Buonocore L, Whitt MA, Rose JK. The minimal conserved transcription stop-start signal promotes stable expression of a foreign gene in vesicular stomatitis virus. J Virol 1996;70(4):2318–23.
[24] Marzi A, Ebihara H, Callison J, et al. Vesicular stomatitis virus-based Ebola vaccines with improved cross-protective efficacy. J Infect Dis 2011;204(Suppl 3):S1066–74.
[25] Marzi A, Menicucci AR, Engelmann F, et al. Protection against Marburg virus using a recombinant VSV-Vaccine depends on T and B cell activation. Front Immunol 2018;9:3071.
[26] Marzi A, Feldmann F, Hanley PW, Scott DP, Gunther S, Feldmann H. Delayed disease progression in cynomolgus macaques infected with Ebola virus Makona strain. Emerging Infect Dis 2015;21(10):1777–83.
[27] Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938;27(3):493–7.
[28] Feldmann F, Shupert WL, Haddock E, Twardoski B, Feldmann H. Gamma irradiation as an effective method for inactivation of emerging viral pathogens. Am J Trop Med Hyg 2019;100(5):1275–7.
[29] Grant-Klein RJ, Antonello J, Nichols R, Dubey S, Simon J. Effect of gamma irradiation on the antibody response measured in human serum from subjects vaccinated with recombinant vesicular stomatitis virus-zaire Ebola virus envelope glycoprotein vaccine. Am J Trop Med Hyg 2019;101(1):207–13.
[30] Emanuel J, Callison J, Dowd KA, Pierson TC, Feldmann H, Marzi A. A VSV-based Zika virus vaccine protects mice from lethal challenge. Sci Rep 2018;8(1):11043.
[31] Garbutt M, Liebscher R, Wahl-Jensen V, et al. Properties of replication-competent vesicular stomatitis virus vectors expressing glycoproteins of filoviruses and arenaviruses. J Virol 2004;78(10):5458–65.
[32] Marzi A, Kercher L, Marceau J, et al. Stat1-Deficient mice are not an appropriate model for efficacy testing of recombinant vesicular stomatitis virus-based filovirus vaccines. J Infect Dis 2015;212(Suppl 2):S404–9.
[33] Menicucci AR, Sureshchandra S, Marzi A, Feldmann H, Messaoudi I. Transcriptomic analysis reveals a previously unknown role for CD8(+) T-cells in rVSV-EBOV mediated protection. Sci Rep 2017;7(1):919.
[34] Mire CE, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Oral and conjunctival exposure of nonhuman primates to low doses of Ebola Makona virus. J. Infect. Dis. 2016;214(suppl 3):S263–S2S7.
[35] Wong G, Qiu X, de La Vega MA, et al. Pathogenicity comparison between the Kikwit and Makona Ebola virus variants in rhesus macaques. J Infect Dis 2016;214(suppl 3):S281–S2S9.
[36] Suder E, Furuyama W, Feldmann H, Marzi A, de Wit E. The vesicular stomatitis virus-based Ebola virus vaccine: from concept to clinical trials. Hum Vaccin Immunother 2018.
[37] Williams KJ, Qiu X, Fernando L, Jones SM, Alimonti JB. VSVDeltaG/EBOV GP-induced innate protection enhances natural killer cell activity to increase survival in a lethal mouse adapted Ebola virus infection. Viral Immunol 2015;28(1):51–61.
[38] Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 2008;454(7201):177–82.
[39] Martinez O, Tantral L, Mulherkar N, Chandran K, Basler CF. Impact of Ebola mucin-like domain on antiglycoprotein antibody responses induced by Ebola virus-like particles. J Infect Dis 2011;204(Suppl 3):S825–32.
[40] Mbala-Kingebeni P, Pratt CB, Wiley MR, et al. 2018 Ebola virus disease outbreak in Equateur province, democratic republic of the Congo: a retrospective genomic characterisation. Lancet Infect Dis 2019;19(6):641–7.
[41] Wawina-Bokalanga T, Vanmechelen B, Marti-Carreras J, et al. Complete genome sequence of a new Ebola virus strain isolated during the 2017 likati outbreak in the democratic Republic of the Congo. Microbiol Resour Announc 2019;8(20).
[42] Geisbert TW, Geisbert JB, Leung A, et al. Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of Ebola virus. J Virol 2009;83(14):7296–304.
[43] Jones SM, Stroher U, Fernando L, et al. Assessment of a vesicular stomatitis virus-based vaccine by use of the mouse model of Ebola virus hemorrhagic fever. J Infect Dis 2007;196(Suppl 2):S404–12.
[44] Dahlke C, Kasonta R, Lunemann S, et al. Dose-dependent T-cell dynamics and cytokine cascade following rVSV-ZEBOV immunization. EBioMedicine 2017;19:107–18.
[45] Farooq F, Beck K, Paolino KM, et al. Circulating follicular T helper cells and cytokine profile in humans following vaccination with the rVSV-ZEBOV Ebola vaccine. Sci Rep 2016;6:27944.
[46] Fathi A, Dahlke C, Addo MM. Recombinant vesicular stomatitis virus vector vaccines for who blueprint priority pathogens. Hum Vaccin Immunother 2019.
[47] Branswell H. WHO, preparing for the worst, makes plans for reduced doses of Ebola vaccine. Stat 2019.
[48] Gsell PS, Camacho A, Kucharski AJ, et al. Ring vaccination with rVSV-ZEBOV under expanded access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report. Lancet Infect Dis 2017;17(12):1276–84.
[49] Kennedy SB, Bolay F, Kieh M, et al. Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. New Eng J Med 2017;377(15):1438–1447.